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Growth Check of Eucalyptus delegatensis (R.T. Baker) Regeneration at High Altitudes in Northeastern Tasmania

D.P. Webb, R.C. Ellis, and P.M. Hallam

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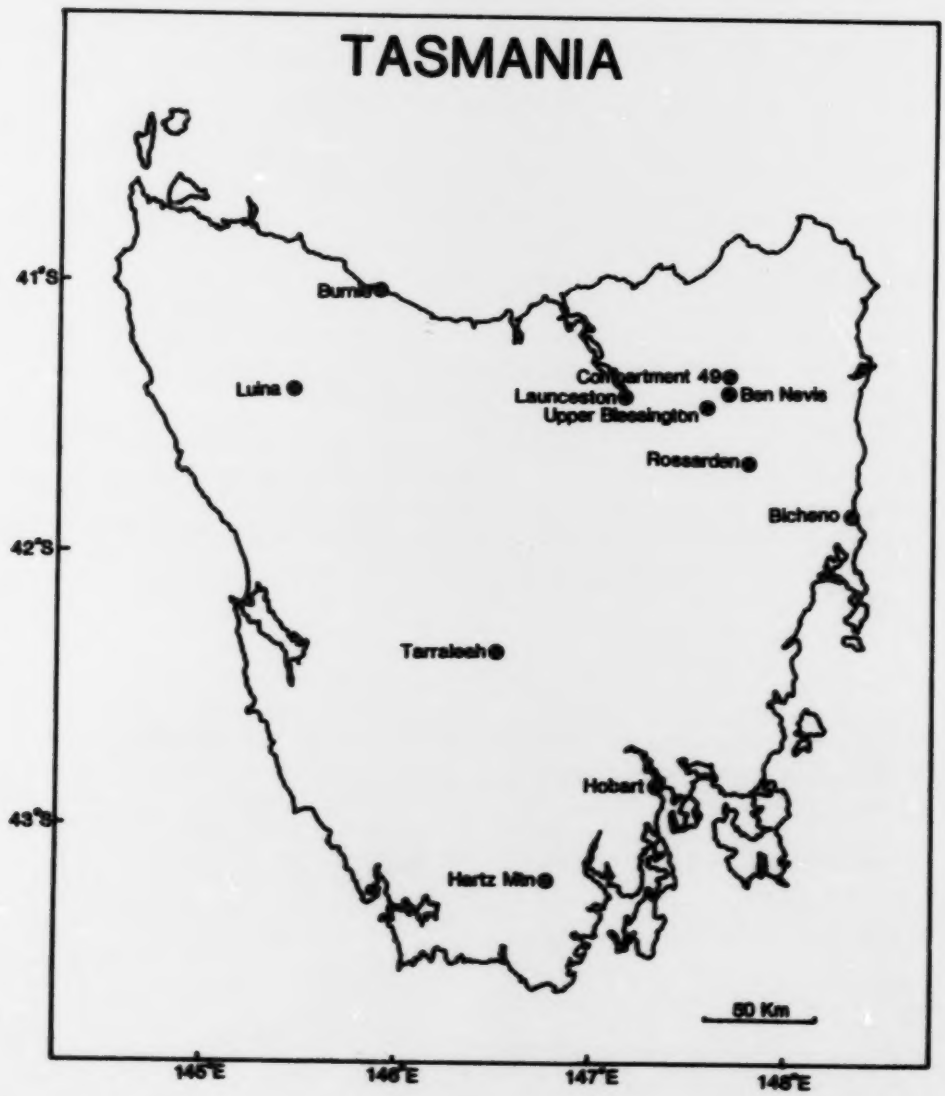

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GROWTH CHECK OF **EUCALYPTUS DELEGATENSIS** (R.T. BAKER) REGENERATION AT HIGH ALTITUDES IN NORTHEASTERN TASMANIA

D.P. WEBB, R.C. ELLIS[1], and P.H. HALLAM[1]

GREAT LAKES FOREST RESEARCH CENTRE

CANADIAN FORESTRY SERVICE

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ABSTRACT

This report outlines the first-year results and experimental design of a three-year study initiated in September 1981, of the ecophysiological factors involved in growth check of **Eucalyptus delegatensis** regeneration at high altitudes in northeastern Tasmania, Australia.

RÉSUMÉ

Le présent rapport communique les résultats de la première année ainsi que le plan d'expériences d'une étude de trois ans entreprise en septembre 1981 sur les facteurs écophysiologiques de l'arrêt de croissance de la régénération d'**Eucalyptus delegatensis** en altitude dans le nord-est de la Tasmanie, en Australie.

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INTRODUCTION

Extensive forests of **Eucalyptus delegatensis** (R.T. Baker) occur in areas of high rainfall in the northeast and northwest of Tasmania on highland plateaus that range in altitude from 600 to 1000 m. The total area involved probably exceeds 500 km^2, of which 80% lies south of Burnie. The principal locations are the Camden Plateau and Paradise Plains in the northeast; the Emu Plains, Borradaile Plains, and Maggs Mountain in the central north; and the Surrey Hills and Hampshire Hills in the northwest. The soils are deep, well drained and derived from granite or basalt, with small areas of permian rocks and dolerite. Not all of this area carries closed eucalypt forest although it is all potentially productive forest land.

All areas have an annual rainfall in excess of 1250 mm. Frosts are frequent in every month of the year except January and February.

There is abundant evidence that on all the areas listed above the climatic climax vegetation is temperate rainforest (Ellis 1964, 1971; Orme 1971). Other evidence, both historical documentary and inferential, indicates that the present forest of E. **delegatensis** over ground vegetation dominated by snow grass (**Poa labillardieri** Steud.) and/or shrubs was established and maintained by regular burning of the original rainforest and the ensuing successional vegetation. Burning, first by aborigines for an unknown period of time, and later by settlers for the purpose of improving grazing for stock, produced multi-aged stands of E. **delegatensis**, usually over a thin sward of grass and low shrubs. The trees were usually of good form, and often reached a height of more than 60 m.

With the cessation of burning by aborigines and a reduction in the practice of grazing in highlands, regular burning ceased. This led to dense growth of snow grass and associated herbs. Invasion of the grass by shrubs appears to have been retarded in many areas by the browsing of native animals. Elsewhere a rainforest understorey developed beneath the eucalypts, and this is often associated with extensive dieback of the eucalypt stands.

Following logging, the understorey is felled by hand or trampled with a bulldozer. After drying for a summer it is burned, usually in late February or early March. Where the amount of debris is adequate, a hot fire is obtained and a satisfactory seedbed is produced. In the absence of an understorey, scarification is sometimes used to prepare a suitable seedbed. Both natural seedfall and aerial sowing are used. Where only a poor burn is obtained, or burning is not practical, irregular regeneration of eucalypts is accompanied by vigorous regeneration of snow grass. In that case, seedlings may go into 'growth check'. Growth check is characterized by poor apical dominance, small, thick, discolored leaves, very slow growth, twisted stems and branches, and absent or necrotic naked buds. Even where a hot fire has produced vigorous regeneration, subsequent development of ground vegetation appears to slow the growth of the eucalypts especially where **Poa** grass is the main component. A similar effect has been observed on limited areas where a high water table favors vigorous growth of Tea tree **(Leptospermum lanigerum)**. In some areas, relatively rapid growth of seedlings may occur following 5-15 years of check; the reasons for this release are obscure. Work on other species has shown that seedlings can be released from growth check by the removal of competing vegetation, especially grass. In one pilot plot with E. **delegatensis**, Orme (1971) ob-

tained rapid initial growth following sterilization of soil in the field with methyl bromide. Growth was checked when grass reinvaded the plot after 2-3 years.

An apparent antagonism of grass and/or associated herbs and shrubs towards a large number of tree species has been noted in many parts of the world. The way in which competing vegetation interferes with tree seedling growth elsewhere has been identified variously as competition for nitrogen and other nutrients, competition for water, production of allelochems by grass, the development of antagonistic microbial populations in the soil, or the occurrence of extreme microclimatic conditions at the surface of the sward.

Historical, and some experimental, evidence indicates that vigorous growth of the trees can be maintained by a program of controlled burning (Ellis et al. 1980). One effect of a single hot burn is to raise the maximum and mean annual soil temperatures at a depth of 10 cm (Ellis 1971). This, and deductions made from the general relationship of check to topographic position and to associated species, indicates that low root temperatures and/or radiation frost may be factors associated with growth check.

The objective of the study was to determine the way in which **Pos** grass interferes with growth of seedlings of **E. delegatensis** so that silviculturally acceptable methods can be developed to overcome the problems of growth check in the field.

Experimental Procedures

In order to define the nature of grass-tree competition more precisely a broad approach to experimentation was undertaken. The preliminary work was designed to examine the possible causes of seedling growth stagnation in order to eliminate minor factors so that research efforts could be directed to the probable main causes. On the basis of results of studies carried out in other ecosystems (Richardson 1953, Sutton 1968, Atkinson 1977, Webb and von Althen 1979, Nambiar and Zed 1980) where removal of competing weed vegetation has a marked effect on tree growth, site preparation treatments were selected that would be expected to elicit a growth response in both established (checked) **E. delegatensis** seedlings and newly transplanted nursery seedlings. The mechanisms whereby grass competes with seedling growth were examined in both field and laboratory experiments that involved investigations of water relations, nutrition, microclimate, root growth and allelopathy.

Experimental procedures and results are reported in the next five sections of this report. A final section is devoted to discussion and conclusions, and recommendations for future work.

SECTION A: EFFECTS OF WEED CONTROL TREATMENTS ON E. **DELEGATENSIS** SEEDLING GROWTH AND SITE FACTORS

Materials and Methods

(a) Single-tree plots

Two study sites were selected in compartment 49, Camden Plateau in northeastern Tasmania. The area described by Ellis (1964) has in the past been noted for the presence of high-altitude dieback. The two sites selected were regenerated to **E. delegatensis** following clearcutting, controlled burning and direct seeding in 1975. In general, growth check in the existing 6-year-old regeneration was most severe on site 1 and was associated with a high density of **Poa** grass that developed after a burn of lower intensity than that obtained on site 2. Eighty-three 6-year-old trees were selected on site 1 and 88 on site 2 covering the range in height of the regeneration. In October 1981, height and maximum crown diameter of the trees were measured and trees were matched in triplets so that there were 26 replicate triplets for site 1 and 28 for site 2. Site preparation treatments, which were randomly assigned to one tree in each triplet, consisted of a) Control--vegetation around the tree was left undisturbed (Fig. A1), b) Herbicide--vegetation around the tree was cut at ground level to a radius of 1.5 m and sprayed with Roundup® on 15 October, 1981 at 5 $ml \cdot L^{-1}$, with Tryquat® on 9 November, 1981 at 5 ml L^{-1} and again with Roundup® on 5 January, 1982 at 6.7 $\cdot ml\ L^{-1}$ (Fig. A2), and c) Manual weeding--vegetation around the tree was scalped at soil surface to a radius of 1.5 m (Fig. A3). Tree heights (one of the best measures of tree release from growth check) were measured at 3 and 6 months and the data were subjected to analysis of variance. On 28 October 1981, five leaves were taken from the 1980-1981 flush of each tree. Within each site they were randomly combined in groups of six trees to give samples of 30 leaves for analysis for N-P-K and dry weight per leaf.

Visual estimations of percentage cover of vegetation on checked and unchecked sites were made from 12 randomly selected plots on each area (Fig. A4).

(b) Planted seedlings

The experiment was designed as a randomized complete block with three treatments and six replications. To each plot (5.5 x 4 m) one of three site preparation treatments as outlined in (a) was assigned. On 27 October 1981, 40 five-month-old **E. delegatensis** seedlings grown in paperpots from locally collected seed (Roses Tier) at the Forestry Commission's nursery in Perth, Tasmania were planted in each plot at a spacing of 0.5 x 0.5 m. Paper surrounding the root systems was removed before planting. Severe frost on or about 23 November and insect damage resulted in the death of many seedlings. Damage was assessed visually and rated on a 0-3 (none, moderate to severe) scale. The experiment was replanted on 1 December, 1981 and seedlings were sprayed with Malathion® to prevent insect attack.

The area was fenced to prevent grazing by native animals. On 26 November 1981 a weather station was installed which consisted of a thermohygrograph, minimum and maximum thermometers, and a rainfall gauge. Tensiometers were installed at 15, 30 and 70 cm depths on each of the control and manually weeded plots and at 15 and 30 cm on the herbicide-treated plots.

Figure A1. Six-year-old E. **delegatensis** seedling on Camden study site: control treatment.

Figure A2. Six-year-old E. **delegatensis** seedling on Camden study site: herbicide treatment.

Figure A3. Six-year-old E. **delegatensis** seedling on Camden study site: manual weed treatment.

At time of planting 36 seedlings were randomly selected and were taken to the laboratory for measurement of initial size parameters. At monthly intervals three seedlings per plot were randomly selected (18 per treatment) and subjected to the following measurements in the laboratory:

1) stem height--length from lowest node to apical bud
2) stem diameter--diameter midway between two lowest nodes
3) dry weight of stem--oven dried at 65°C for 48 hr
4) dry weight of leaves--oven dried at 65°C for 48 hr
5) dry weight of roots--oven dried at 65°C for 48 hr
6) number of lateral roots--1st and 2nd order
7) root length--length of longest root
8) number of leaves
9) leaf areas--initially calculated from a regression of area and weight of an image of leaves on photocopy paper

$$\text{leaf area } (cm^2) = -0.088767 + 124.1139 \text{ weight (g)}$$
$$r = 0.99$$

and later by a regression of leaf area on sum of length of leaf (L) x width (W) over all leaves on each plant.

$$\text{leaf area } (cm^2) = 0.547 + 0.752596 (L \times W)(cm^2)$$
$$r = 0.99$$

Measurements common to a) and b)

At each time of sampling, soil cores were taken from each plot at 5-10 cm depth and were weighed and dried to determine gravimetric soil moisture. Soil temperature was also recorded at the 10 cm depth with a thermocouple probe.

Bulk soil samples were obtained from a soil pit between levels of discontinuity corresponding to A, B and C horizons (Table A1), and soil moisture-matric suction curves were determined on those samples by means of a pressure plate apparatus and standard techniques (Richards 1965) (Table A2). Planted seedlings or established trees from the treated areas were selected at intervals during the summer for measurement of water relations parameters. Measurements made from sunrise to sunset over a 2- to 3-day period to establish diurnal patterns consisted of:

1) leaf water potential--measured in a pressure chamber
2) stomatal resistance and leaf temperature--measured by means of a diffusive resistance porometer
3) osmotic potential of expressed sap from leaf discs frozen (approx. -35°C) in the field by a stream of liquid freon--measured by the dewpoint method on a Wescor psychrometer
4) light intensity--measured by a Kipp and Zonen solarimeter
5) atmospheric humidity deficits--measured with an Assman psychrometer

Details of calibration and use of instruments are outlined in Section D.

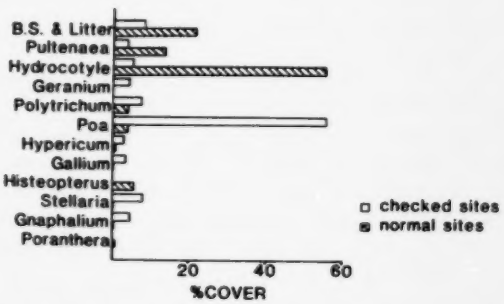


Figure A4. Distribution of understorey species.

Table A1. General description of Camden soil.

Profile depth (cm)	Horizon	Field texture	% Gravel[a]
0-10	A	gritty clay loam	19.2
30-50	B	gritty light clay[b]	4.7
60-90			79.4
>70	C	decomposing granite	
>100			153.2

[a] particle size greater than 2 mm and expressed as percentage of oven-dry soil.

[b] Soil color. B. horizon: mottled 7.5 YR 5/6 and 7.5 YR 5/8 (Munsell Color Code).

Results

(a) Single-tree plots

*E. **delegatensis*** trees showed increased height growth ($P \leq 0.05$) at both 3 and 6 months after removal of competing vegetation by manual weeding (Table A3). At 6 months, increased height growth of 154% and 161% over controls was obtained on site 1 and site 2, respectively. The herbicide treatment showed a slight but non-significant trend towards improved growth. Application of Roundup® followed by Tryqual® controlled the competing vegetation and prevented regrowth of **Poa** grass over the experimental period. No residual phytotoxic effects of the herbicide on existing eucalypts was observed. The fact that the herbicide treatment did not result in a marked improvement in height growth suggests that factors other than direct physical competition may be operative in the short term.

Analysis of soil and leaf samples taken at the time of treatment establishment on 28 October 1981 indicated little difference in soil nutrient status between sites but highly significant differences in foliar concentrations and contents of N, P, and K (Table A4). After approximately 4 months, differences were observed in the amount of available ammonium and nitrate nitrogen in the soil of the three treatments (Table A5).

Table A2. Relationship between soil matric suction and percentage soil moisture for Camden soil at various depths.

	Moisture (%)			
	Depth (cm)			
Pressure (bars)	0-10	30-50	60-90	> 100
- 0.33	30.72	23.78	14.60	7.93
- 0.50	30.57	22.05	13.59	7.06
- 1.00	28.73	20.95	13.06	6.12
- 4.00	23.86	16.69	7.75	4.05
-10.00	18.22	12.15	4.83	2.55
-15.00	16.68	10.58	3.98	2.10

Curve fitted is:

$Y = bX^m$ where Y = % moisture

X = pressure (bars)

0-10 cm m = -0.1637, b = 27.31, r = -0.97

30-50 cm m = -0.2053, b = 19.82, r = -0.98

60-90 cm m = -0.3473, b = 11.13, r = -0.98

>100 cm m = -0.3431, b = 5.75, r = -0.99

Table A3. Site preparation and tree height growth (m): single-tree plots.

Treatment	Site 1		Site 2	
	3 Months	6 Months	3 Months	6 Months
Control	0.062[a]	0.181[c] (n=20)	0.063[a]	0.136[c] (27)
Herbicide	0.057[a]	0.198[c] (20)	0.064[a]	0.158[c] (27)
Man. Weed	0.115[b]	0.279[d] (20)	0.089[b]	0.219[d] (27)

$n \geq 25$ at 3 months

Similar letters in vertical columns indicate means that are not significantly different at $P \leq 0.05$.

Table A4. Selected chemical properties of Camden soil and foliage nutrient levels of *E. **delegatensis*** trees collected from site 1 and site 2 single-tree plots on 28 October 1981.

Soil

Site	Total organic carbon (%)	Total nitrogen (%)	Ammonium nitrogen (ppm)	Nitrate nitrogen (ppm)	pH
1	6.5[a]	0.43	18.7	2.5	5.6
2	6.0	0.43	11.4	1.0	5.6

Foliage

Site	Mean leaf weight (g)	Concentration (%)			$mg \cdot leaf^{-1}$		
		N	P	K	N	P	K
1	0.69[b]	1.24	0.095	0.40	8.49[b]	0.65[b]	2.74[b]
2	0.39	1.28	0.107[b]	0.46[b]	4.94	0.41	1.80

[a] 0-10 cm soil samples

[b] Data significantly different from those of second site ($P \leq 0.05$).

Table A5. Effects of site preparation treatments (planted seedlings) on soil nitrogen.

Treatment	Ammonium nitrogen (ppm)	Nitrate nitrogen (ppm)	pH
Control	4.9[a]	0.0	4.8
Herbicide	17.8	6.9	5.1
Manual weed	25.4	11.9	4.9

[a]Soil samples from 0-5 cm depth on 24 February 1982.

Changes in water relations parameters were measured from sunrise to sunset over 3-day periods (21-23 December 1981 and 26-28 January 1982 for the manually weeded and control treatments and 25-26 February 1982 for the three site preparation treatments).

Leaf and air temperatures followed the expected patterns with a midday peak during the December sampling period closely following the diurnal pattern of light intensity and absolute humidity deficits (Fig. A5, A6, A7). Shoot xylem pressure

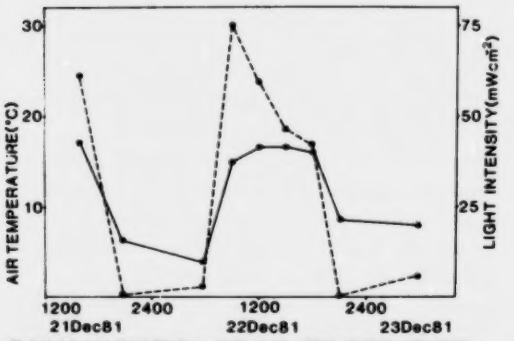


Figure A5. Diurnal variation in air temperature (solid line) and light intensity (broken line).

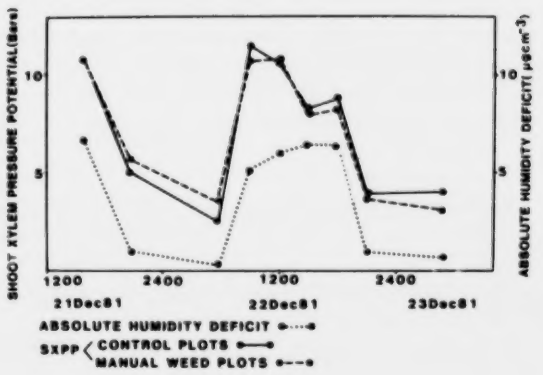


Figure A6. Diurnal variation in absolute humidity deficits (dotted line) and shoot xylem pressure potential in control (solid line) and manually weeded plots (broken line).

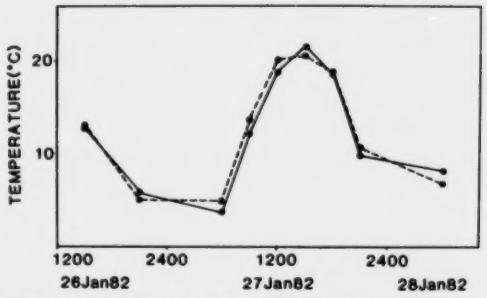


Figure A7. Diurnal variation in air temperature (solid line) and leaf temperature (broken line).

potential followed a similar pattern (Fig. A6, A8, Table A6). No differences in water relation parameters were obtained between treatments. Similar patterns in water relations parameters were observed in the January and February sampling (Table A7, A8) and again no differences were observed between treatments.

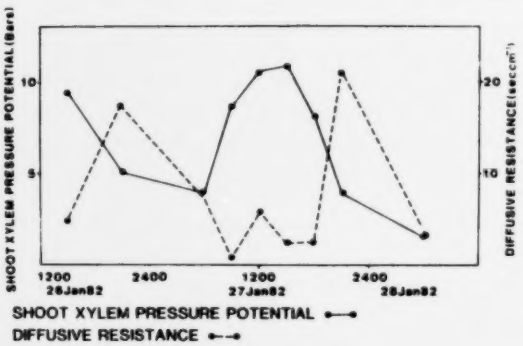


Figure A8. Diurnal variation in shoot xylem pressure potential (solid line) and leaf diffusive resistance (broken line).

Examination of turgor pressure and stomatal resistance data (Tables A6, A7 A8) indicates that, in general, water was not limiting plant growth on any of the site preparation treatments.

The only parameters showing differences between treatments were soil water potential (Fig. A9), soil temperature (Fig. A10) and available nitrogen (Table A5). The marked increase in these parameters in the manually weeded treatments corresponded with increased height growth of existing E. **delegatensis** regeneration.

(b) Planted seedlings

Removal of competing vegetation by manual weeding resulted in marked stimulation of growth of E. **delegatensis** seedlings over a 101-day growing period (Fig. A11). Similarly, removal of competing vegetation by herbicide application resulted in significant but less marked stimulation in growth. Shoots were stimu-

Table A6. Effects of site preparation treatments on selected water relations parameters, 21-22 Dec. 1981.

Time	Date	Treatment	Ψ Leaf (bars)	Ψ π (bars)	Turgor (bars)	Ψ Soil (bars)
1600	21 Dec.	C[a]	-12.7[b]	-12.3	0.4	-0.08
		MW	-10.3	- 9.5	0.8	-0.37
2040	21 Dec	C	- 4.7	- 9.9	5.2	
		MW	- 5.5	-11.7	6.2	
0540	22 Dec	C	- 2.1	-10.8	8.8	-0.20
		MW	- 4.3	-11.0	6.8	-0.09
0900	22 Dec	C	-11.9	-13.7	1.7	
		MW	-10.9	-10.3	-0.7	
1200	22 Dec	C	-11.9	-13.7	1.8	
		MW	-10.1	- 8.6	-1.5	
1500	22 Dec	C	- 8.3	-13.2	4.9	-0.14
		MW	- 7.3	-	-	-0.17

[a] C - control, MW - manual weed
[b] 1 bar = 100 kPa

Table A7. Effects of site preparation treatments on selected water relations parameters, 26-28 January 1982.

Time	Date	Treatment	Ψ Leaf (bars)	Ψ π (bars)	Turgor (bars)	Ψ Soil (bars)	Leaf diffusive resistance R (sec·cm^{-1})
1500	26 Jan	C[a]	- 9.4[b]	- 9.2	-0.2	-0.54	4.80
		MW	- 9.7	- 9.0	-0.7	-0.45	3.39
0600	27 Jan	C	- 4.3	- 9.7	5.4	-0.44	6.52
		MW	- 3.0	-10.7	7.7	-0.45	6.93
0900	27 Jan	C	- 8.5	- 8.8	0.3		0.57
		MW	- 9.2	-12.1	2.9		0.95
1200	27 Jan	C	-10.6	-12.8	2.2		5.70
		MW	-10.8	-13.1	2.3		2.99
1500	27 Jan	C	-10.6	-10.6	0.0	-0.41	2.31
		MW	-10.7	-13.0	2.3	-0.26	1.80
1800	27 Jan	C	- 8.4	- 7.7	-0.7		2.45
		MW	- 7.8	-15.2	7.4		2.07
2100	27 Jan	C	- 4.3	- 9.7	5.4		20.91
		MW	- 3.5	-13.9	10.5		11.26
0600	28 Jan	C	- 1.1	- 8.2	7.1	-0.58	3.35
		MW	- 1.2	-10.9	9.7	-0.14	6.01

[a] C - control, MW - manual weed.
[b] 1 bar = 100 KPa

Table A8. Effects of site preparation treatments on selected water relations parameters, 25-26 February 1982.

Time	Date	Treatment	Ψ Leaf (bars)	Ψ π (bars)	Turgor (bars)	Ψ Soil (bars)	Leaf diffusive resistance R (sec·cm^{-1})
1500	25 Feb	C[a]	- 9.4[b]	-14.3	4.9	- 0.46	6.61
		H	-10.0	-14.1	4.1	- 0.30	5.20
		MW	-10.3	-15.5	5.2	- 0.46	5.05
0700	26 Feb	C	- 1.5	-14.7	13.2		12.21
		H	- 1.3	-12.1	10.8		10.67
		MW	- 1.7	-14.9	13.2		8.6

[a] C - control, MW - manual weed, H - herbicide
[b] 1 bar = 100 KPa

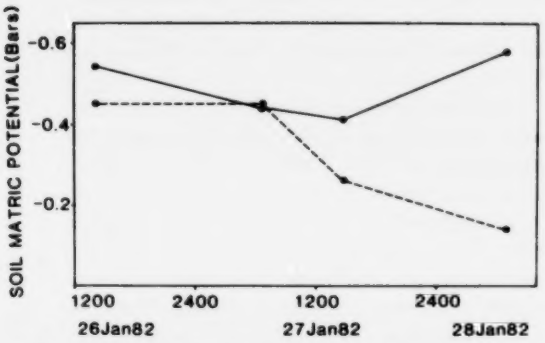


Figure A9. Variation in soil moisture measured at 5-10 cm depth in control (solid line) and manually weeded plots (broken line).

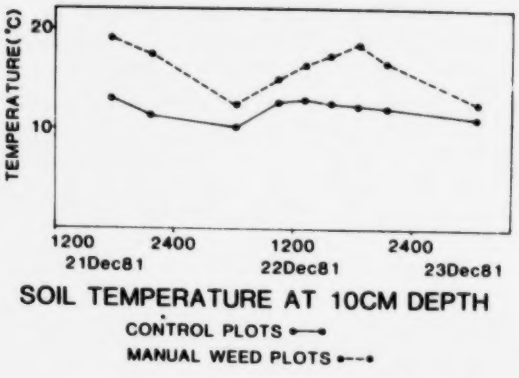


Figure A10. Variation in soil temperature measured at the 10 cm depth in control (solid line) and manually weeded plots (broken line).

lated to a greater extent than roots, to give lower root:shoot ratios for treated seedlings. No phytotoxic effects of herbicide application were observed on planted seedlings. During the growth period mean daily temperatures varied between 10 and 25°C, with minimum temperatures falling to less than 0°C on several occasions during the growing season (Fig. A12). The treatments caused marked differences in soil temperature and moisture (Table A9), and this was also observed in the single-tree plots. Manual weeding increased soil temperature at the 10 cm depth by approximately 5°C, and soil water potential was increased over that of the controls.

Soil nutrient levels were markedly altered by the treatments. Available ammonium and nitrate nitrogen at the 0-5 cm depth were higher in the manually weeded treatment than in the control (see Section C).

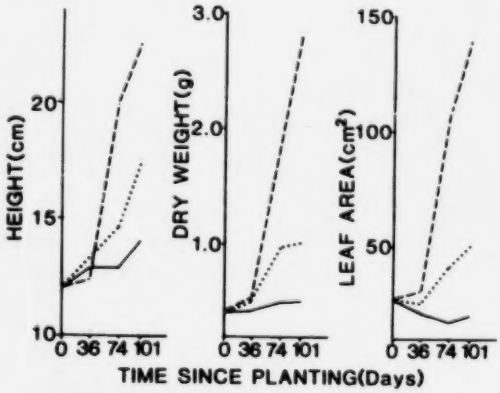


Figure A11. Growth of E. **delegatensis** seedlings in control plots (solid line), herbicide plots (dotted line) and manually weeded plots (broken line) during 1981-1982 on the Camden Plateau.

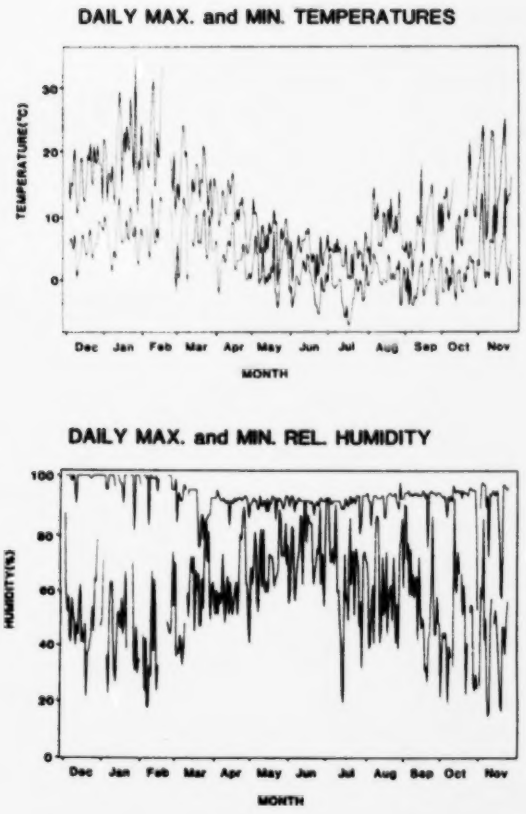


Figure A12. Daily maxima and minima screen temperatures and relative humidity on Camden Plateau (fenced plot).

Water relations parameters of randomly selected seedlings (six per treatment) were measured at 3:00 p.m. and before dawn on 11 and 12 February 1982. Decreased soil water potential in the control plots resulted in seedling leaf water potentials of over -30 bars (-3000 KPa) during the maximum stress period and stomatal resistances of approximately 70 $sec \cdot cm^{1}$, which indicated stomatal closure (Table A10). Seedlings in the manually weeded and herbicide treatments showed favorable water relations with leaf water potentials of -11 to -12 bars (-1100 to -1200 KPa) and low stomatal resistances (Table A10).

Predawn measurements indicated nearly complete recovery from water stress in seedlings in the manually weeded and herbicide treatments by equilibrating to approximately -4 bars (-400 KPa) (Table A10). In contrast, predawn leaf water potentials of seedlings in control plots recovered somewhat, but remained at a relatively high -15 bars (-1500 KPa).

Seedlings in all treatments exhibited negative turgor during maximum stress periods, but only in the control was this condition maintained overnight (Table A10).

Discussion

The water relations data obtained in this experiment indicate that water stress was an important factor limiting growth of planted seedlings in control plots. During summer weather conditions in the relatively high rainfall area of the Camden Plateau (>1250 mm), seasonal distribution of rainfall (see Table E1b) can result in a substantial reduction in soil moisture enhanced by competing vegetation to levels severe enough to affect markedly the growth of shallow rooted E. **delegatensis** seedlings.

In contrast, the same weather conditions were not severe enough to cause any measurable treatment differences in water relations of larger, more deeply rooted, established trees. If soil water was limiting growth in this case it would be expected that, in general, both weed control treatments would elicit similar growth responses, or that a larger growth response to the herbicide treatment would result from the increased soil moisture

Table A9. Effects of weed control treatments on soil temperatures and water potential (10 cm depth).

Treatment	5 Jan 82		15 Jan 82		11 Feb 82	
	Temp. (°C)	Ψ Soil (bars)	Temp. (°C)	Ψ Soil (bars)	Temp. (°C)	Ψ Soil (bars)
Control	15.4	- 0.61[a]	17.5	- 0.45	17.2	- 2.50
Herbicide	17.0	- 0.19	19.2	- 0.03	19.0	- 0.67
Man. weed	19.6	- 0.10	22.8	- 0.20	22.0	- 1.38

[a] 1 bar = 100 KPa

Table A10. Effects of weed control treatments on selected water relations parameters, 11-12 February 1982.

Time	Date	Treatment	Ψ Leaf (bars)	Ψ π (bars)	Turgor (bars)	Ψ Soil (bars)	Leaf diffusive resistance R ($sec \cdot cm^{-1}$)
1530	11 Feb.	C[a]	< -30.4[b]	-19.0	< -11.4	- 2.50	69.82
		H	-11.6	- 7.4	- 4.2	- 0.67	6.99
		MW	-10.7	- 6.0	- 4.7	- 1.38	7.31
0630	12 Feb.	C	-14.8	-12.4	- 2.3		12.44
		H	- 4.1	- 7.0	3.0		13.48
		MW	- 4.6	- 4.9	0.3		4.77

[a] C - Control, H - herbicide, MW - manual weed.
[b] 1 bar = 100 KPa

retention as a result of mulching of the dead vegetation. Obviously, other factors are involved in the growth response of established trees to site preparation treatments.

The observed difference in soil temperature of up to 5°C between treatments may contribute to the growth differences observed. If we assume that root growth at temperatures between 15.4 to 17.5°C in the control treatment is less than optimum, increases of approximately 2°C in the herbicide treatment and over 5°C in the manually weeded treatment could contribute to the increased growth observed. It is not possible at this stage to determine what effect a 2-5°C increase in soil temperature would have on overall seedling growth of **E. delegatensis** because base data are lacking. Results from other species suggest that major growth differences would be expected from such a rise in soil temperature. It would appear that the increased growth of the more deeply rooted, established regeneration in response to weed control treatments could be related to the increased soil temperature of the rooting zone. Differences in nutrition observed between treatments also affect growth. Nutrition, soil temperature, and soil moisture interact and may change in relative importance with the stage of development of the seedlings.

It is possible that the addition of herbicide could have affected growth directly and is responsible for the differences observed between the two site preparation treatments. Of the herbicides used, only Tryquat has any reported residual nature and no phytotoxic effects were noted on planted seedlings and established trees.

It is also possible that growth differences result from the removal of an allelochem produced from competing vegetation that was removed during site preparation treatments. The effects of nutrition and of the possible presence of allelochems on **E. delegatensis** seedling growth are examined in Sections B and C.

The deforested areas of the Camden Plateau are subject to frequent unseasonable frosts (see Table E1a). On 27 October 1981, the planted seedlings suffered severe frost damage from a -4°C frost measured at ground level. Plants obtained from the Forestry Commission's nursery at Perth, grown from a local seed source (Roses Tier), had approximately 3 weeks to adapt to the site prior to this frost. Therefore, lack of conditioning and seed source were not factors that might have increased susceptibility to frost. Frost and insect damage assessed on 26 November 1981, and survival data obtained on 11 February 1982, showed increased damage in control and herbicide treatments (Tables A11, A12). The area was replanted on 1 December 1981. On 6 and 7 March 1982, a -2°C frost (screen temperature) occurred during rapid vegetative growth and caused high mortality. Damage was not assessed at this time because of lack of regrowth and difficulties in assessing the viability of trees.

Table A11. Damage appraisal of planted seedlings exposed to various site preparation treatments, Camden 26 November, 1981.

Treatment	Frost damage	Insect damage
Control	75.3[a]	47.5
Herbicide	86.3	55.5
Manual weed	67.5	10.2

[a] Damage was assessed on the scale 0 no damage, 1 slight, 2 moderate, 3 severe on three replications of 40 seedlings per treatment. Maximum possible rating is 120.

The susceptibility of E. **delegatensis** seedlings to frost damage, particularly during the active vegetative growth phase, can have marked effects on seedling growth and can contribute to growth check. The role of frost in growth check of E. **delegatensis** seedlings requires further experimentation (See Section E).

Table A12. Survival of planted seedlings on the Camden Plateau assessed on 11 February 1982.

	Planting date	
Treatment	27 Oct 1981	1 Dec 1981
Control	34.4[a]	59.9
Herbicide	53.8	90.1
Manual weed	90.6	94.6

[a] Data based on three replications of 40 seedlings per treatment.

SECTION B: PLANT COMPETITION: STUDIES OF TREE SEEDLING/GRASS INTERACTION

Interspecies Competition

Experiments were undertaken to examine the nature of grass-tree interaction under semi-controlled conditions. Initial experiments were designed to separate physical from chemical competition under conditions of adequate nutrition and water (Webb and von Althen 1979).

On 7 November, 1981 **Poa** grass **(Poa labillardieri)** and **E. delegatensis** seedlings were planted in 11 cm diameter x 10 cm high plastic pots containing a commercially prepared sterilized peat-sand potting mixture amended with N, P and K plus micronutrients. Seedlings were grown in the glasshouse of the Agricultural Sciences Department at the University of Tasmania for approximately 4 weeks to allow for initial adaptation and establishment. Glasshouse temperatures ranged from a mean daily minimum of 17°C to a mean daily maximum of 34°C. Seedlings were then selected for uniformity in height and branching, and grass was selected for uniformity in biomass, prior to allocation to treatments. As a result, approximately 50% of the plants grown were discarded. Six pots were randomly assigned to each of the following treatments:

1) seedling alone in pot
2) grass alone in pot
3) seedling and grass in same pot
4) seedling and seedling in same pot
5) single seedling watered with leachates obtained from seedling grown in pot as in 1) above
6) Single seedling watered with leachates obtained from grass growing in pot as in 2 above
7) single seedling watered with leachates from bare soil
8) grass alone watered with leachates obtained from seedling growing in pot as in 1) above
9) grass alone watered with leachates obtained from grass growing in pot as in 2) above
10) grass alone watered with leachates from bare soil

Aliquots (50 ml each) of each leachate were applied twice weekly.

At weekly intervals, seedling shoot heights were measured from the soil surface to the apical bud. At week 11 all plants were harvested and the following growth parameters were measured:

1) stem height--distance from lowest node to apical bud
2) stem diameter--diameter midway between two lowest nodes
3) number of lateral branches longer than 10 mm
4) length of lateral branches
5) dry weight of stem, leaves and roots --oven-dried at 65°C for 48 hr
6) number of lateral roots--primary and secondary laterals
7) length of longest root
8) number of leaves
9) leaf areas (as in Section A)

Total dry weight, root:shoot ratio on a dry weight basis and index of apical dominance ($\frac{\text{stem height}}{\text{total length of lateral shoots}}$) were calculated.

Results and Discussion

Significant differences in height growth were observed between seedlings grown alone and seedlings grown with either grass or another seedling (Fig. B1, Table B1). However, no significant differences were observed between seedlings grown with grass and seedlings grown with another seedling (Fig. B1). The application of leachates from grass or seedlings to seedlings or grass showed no significant differences over bare soil leachates similarly applied to seedlings or grass (Table B1). It would appear from these results that **Poa** grass interferes with seedling growth by physical competition rather than by the production of readily soluble allelochems produced from living plants inhibitory to seedling growth.

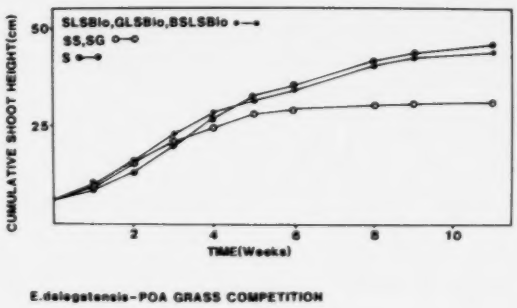


Figure B1. Mean cumulative shoot height of E. **delegatensis** seedlings in various treatments to examine effects of E. **delegatensis--Poa** grass competition. S - seedling grown alone; SS - seedling grown with another seedling; SG - seedling grown with grass; SLSBio - seedling leachate applied to seedling used as bioassay; GLSBio - grass leachate applied to seedling used as bioassay; BSLSBio - bare soil leachate applied to seedling used as bioassay.

Allelopathy

In the previous experiment, no inhibitory effects of leachates obtained from living **Poa** grass on the growth of E. **delegatensis** seedlings could be demonstrated. It is possible that allelochems can be produced from decomposition of vegetation associated with checked seedlings in the field.

a) Established seedlings and soil leachates

An experiment was undertaken to examine the effect of leachates from soil blocks obtained from areas (Camden, site 2) where natural regeneration either was or was not in a state of growth check. Twelve undisturbed soil blocks (30 cm x 25 cm x 10 cm deep) were obtained from each area showing growth check or normal growth and were placed in plastic basins and incubated in the greenhouse with regular watering to prevent moisture stress. At weekly intervals leachates (200 ml) were obtained and applied to six replicates of single seedlings or grass as outlined in the previous experiment. Treatments consisted of:

1) checked leachates--seedling
2) checked leachates--grass
3) unchecked leachates--seedling
4) unchecked leachates--grass

Seedling heights were recorded at weekly intervals. After 11 weeks' growth in the glasshouse environment the experimental seedlings and grass were harvested and measured as in the previous experiment.

Visual estimates of percentage cover by each species on each soil block obtained from checked and unchecked areas were made at the end of the experiment.

b) New germinants and soil/grass leachates

Newly germinated seeds of E. **delegatensis**, with radicles just emerging, were placed in petri dishes on filter paper overlying 3 mm of vermiculite. Five seeds were placed in each of 28 petri dishes.

Table B1. Effects of **Poa** grass competition on E. **delegatensis** height growth.

Treatment	Mean seedling height (cm)										
	0	1	2	3	4	5	6	8	9	10	11
Seedling grown alone	6.1	8.8	13.3	19.7	27.0	32.5	35.2*[b]	41.9[b]	43.6[b]	44.2[b]	45.7[b]
Seedling + seedling	6.5	10.1	14.4	19.4	23.2	24.9	31.5[a]	29.3[a]	30.0[a]	30.6[a]	30.7[a]
Seedling + grass	6.3	9.9	15.6	21.1	24.4	26.2	28.8[a]	29.9[a]	30.6[a]	31.5[a]	31.2[a]
Leached seedling	6.2	9.4	15.2	20.6	25.2	26.8	28.6[a]	31.5[a]	33.2[a]	34.2[a]	34.0[a]
Seedling leachate seedling bioassay	6.3	10.0	15.9	22.8	29.5	33.4	35.8[b]	40.9[b]	42.5[b]	45.1[b]	46.1[b]
Grass leachate seedling bioassay	6.0	10.3	16.1	23.3	28.4	31.48	34.0[b]	40.2[b]	42.7[b]	44.7[b]	45.2[b]
Bare soil leachate seedling bioassay	6.0	9.9	15.6	21.0	26.6	30.5	32.7[b]	37.0[b]	39.4[b]	41.0[b]	41.8[b]

*Data are means of six measurements per treatment. From week 6 onward means with similar letters are not significantly different at $P \leq 0.05$. No data were obtained for week 7.

From two sites which were dominated by grass and in which eucalypt seedlings were in check, collections were made of surface litter plus grass, soil at 0-7.5 cm depth containing dense grass roots, and soil at 7.5-15.0 cm depth. Samples were comminuted and passed through a 0.5 cm sieve.

Extracts were made in each case by shaking 20 g of material with 100 ml distilled water for 16 hr. A 5 ml portion of filtered extract was added to each petri dish: four dishes per extract of litter (soil from 0-7.5 cm depth and soil from 7.5-15.0 cm depth from each of the two sites) and four distilled water controls were used.

Lengths of radicles and shoots of each germinant were measured at intervals for 11 days.

Results and Discussion

a) Although marked differences in species composition were observed between checked and unchecked areas (Fig. A4) no significant differences were found in various growth parameters between leachate treatments (Table B2). Soil blocks from areas showing normal growth had **Hydrocotyle** and **Pultenaea** as the dominant species whereas checked areas were dominated by **Poa** grass. As in the previous experiment no results were obtained to

support the view that grass-tree competition results from production of allelochems.

b) The mean rate of increase in length of both radicle and shoot between each measurement was greater for germinants treated with each extract than for those treated with distilled water. Only small differences were found among extracts from the two sites: hence, data from the sites are combined in Figure B2. In no case was growth inhibition observed.

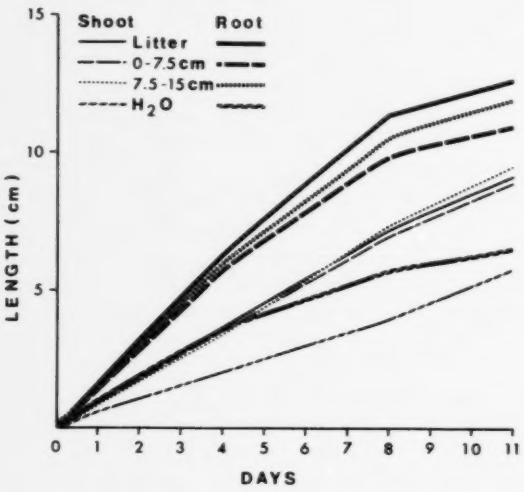


Figure B2. Effects of various aqueous soil and litter extracts on growth of E. **delegatensis** germinants.

Although it is extremely difficult to prove the absence of allelochems, it is considered that if allelopathy was a major factor in grass-tree competition the experimental design would have indicated the effect although seasonal effects of decomposing vegetation were not examined. However, the lack of any significant effects leads one to conclude that the mechanism of **Poa** grass-tree interaction probably involves physical competition rather than chemical competition such as allelopathy.

Root Competition

A further experiment was undertaken to examine the effects of grass competition on E. **delegatensis** seedling root growth. Root observation boxes with two 58 cm x 30 cm glass panels 7 cm apart were evenly packed with commercial sterilized sand-peat mixture as described previously. Five replicate boxes each were planted in the following arrangements:

1) grass plants alone
2) seedling alone
3) seedling with grass
4) seedling with seedling

The plants were watered regularly to prevent moisture stress and the boxes were slanted on an angle of approximately 20° so that the roots would contact the glass panel. Light was excluded from the root zone by wrapping the boxes in black plastic. After approximately 10 days' adaptation to the glasshouse environment, and when roots appeared on the glass panel, measurements were begun. Seedling height was measured about every 2 days. Root elongation was measured from root tracings with a felt marker on the glass plate at daily intervals. After approximately 16 days of growth, by which time rooting patterns had been established and roots had reached the bottom of the boxes, the measurements were suspended. Root observation boxes were dismantled by sliding the glass panels out of the wooden supports and pushing a nail board (spacing 5 x 5 cm) through the soil medium. The soil was then washed away from the roots with a fine stream of water and the roots

Table B2. Effects of soil leachates from soil blocks obtained from areas on the Camden Plateau showing seedling growth check or normal growth.

Treatment	Mean seedling height (cm)										
	Time (weeks)										
	0	1	2	3	4	5	6	8	9	10	11
Leachates from 'checked' soil block applied to seedling bioassay	8.0[a]	11.8	16.0	21.1	25.7	27.0	28.2	32.0	33.7	34.3	34.6
Leachates from 'unchecked' soil blocks appled to seedling bioassay	8.1	12.4	17.5	23.9	27.7	29.7	30.7	33.9	34.5	35.7	35.8

[a] Data are means of six seedlings per treatment. From week 8 onward, lines connect means that are not significantly different at $P \leq 0.05$. No data were obtained for week 7.

Table B3. Root observation boxes: mean root dry weight distribution with depth.

Sample depth (cm)	Seedling alone	Seedling + seedling	Seedling + grass seedling roots	Grass alone	Grass + seedling grass roots
0-5	0.24^{c}	0.17^{c*}	0.16^{c}	3.46^{b}	13.90^{a}
5-10	0.10^{b}	0.06^{b}	0.04^{b}	0.69^{b}	1.84^{a}
10-15	0.046	0.053^{c}	0.028^{c}	0.415^{b}	0.614^{a}
15-20	0.070^{b}	0.040^{b}	0.026^{b}	0.306^{b}	0.387^{a}
20-25	0.050^{b}	0.033^{b}	0.013^{b}	0.282^{a}	0.253^{a}
25-30	0.038^{b}	0.026^{b}	0.012^{b}	0.226^{a}	0.181^{a}
30-35	0.029^{b}	0.018^{b}	0.009^{b}	0.242^{a}	0.120^{b}
35-40	0.039^{b}	0.012^{b}	0.006^{b}	0.191^{a}	0.108^{ab}
40-45	0.035^{b}	0.009^{b}	0.004^{a}	0.189^{a}	0.074^{ab}
>50	0.021	0.017	0.018	0.181	0.044
Total root dry wt	0.70^{c}	0.45^{c}	0.32^{c}	6.32^{b}	17.58^{a}

Data in columns with the same letter are not significantly different at $P \leq 0.05$.
*Mean seedling root dry weight

were held in position by the nail board (Fig. B3). Normal growth parameters were measured on the seedlings, as outlined previously, and above-ground biomass of the grass was measured. Root dry weight was determined for each plant at successive 5 cm depths.

Results and Discussion

Seedling height growth was greatest when seedlings were grown alone, it was reduced when seedlings were grown with a second seedling, and it was further reduced when seedlings were grown with grass (Table B4) although differences were not statistically significant. Grass leaf biomass did not differ among treatments. In general, grass root elongation rates were significantly greater (four to six times) than seedling root elongation rates (Fig. B4). Similarly, seedling root elongation was reduced when seedlings were grown with other seedlings and further reduced when they were grown with grass. Grass root elongation was markedly stimulated in the presence of E. **delegatensis** seedlings (Fig. B4). Similarly, grass root dry weight in the 0-5 cm depth zone was markedly increased (Table B3). The mechanism whereby E. **delegatensis** seedlings enhance root growth of **Poa** grass is not known and requires further study. A similar effect was noted when **Poa** was grown in pots in mixture with **Themeda australis** (Groves et al. 1973). If the same effect is operative in the field it would indicate that **Poa** grass is extremely competitive with eucalypt seedlings.

Figure B3a. Root observation boxes used for measuring root elongation. Note white lateral roots of **Poa** grass.

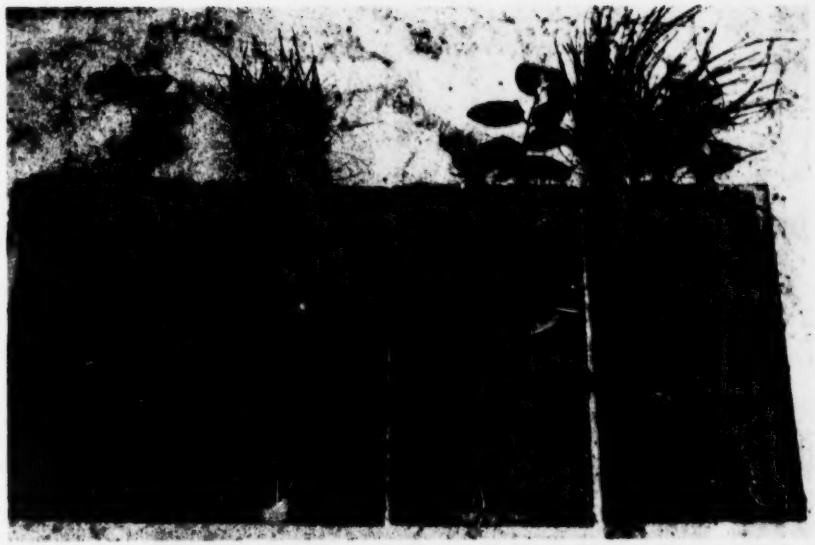
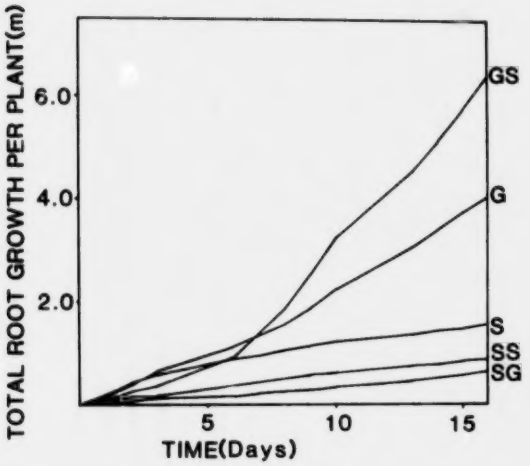


Figure 83b) Nailboards showing root distribution of E. **delegatensis** seedlings and **Poa** grass grown alone and in combination with each other in root observation boxes.



ROOT GROWTH OF E.delegatensis SEEDLINGS AND POA GRASS

Figure 84. Root elongation of E. **delegatensis** seedlings grown alone (S), with another seedling (SS), and with grass (SG), and root elongation of **Poa** grass grown alone (G), or with a seedling (GS) as measured in root observation boxes.

SECTION C: NUTRITION: EFFECTS OF SOIL AMENDMENT ON SEEDLING GROWTH AND ON SOIL NUTRIENT LEVELS

In a detailed study of the interaction of **Acer pseudoplatanus** L. and the grass **Lolium perenne** L., Richardson (1953) found that the presence of grass depressed the rate and reduced the extent of root growth of the trees. A deficiency of N reduced the size and growth of tree roots but increased the elongation of grass roots, thereby increasing the competitive ability of the grass.

Numerous studies have shown that both the equilibrium level of mineral nitrogen and the rate of mineralization of nitrogen in grassland soils are lower than those normally encountered in agricultural and many forest soils. The levels are particularly low in very old, natural grassland (O'Connor et al. 1962, Robinson 1963, Iheron 1963) in comparison with those in agricultural leys (Richardson 1938, Chase et al. 1968) or in secondary successional grassland (O'Connor et al. 1966, Ellis 1974).

Nitrogen is taken up by grass mainly or entirely in the form of ammonium (Chase et al. 1968) whereas trees may take up either ammonium or nitrate or a combination of the two (see, for instance, Moore and Keraitis 1971).

(a) Seedling Pot Studies

Objective

To determine the relationship between the supply of N and seedling growth in soil from the experimental plots as a guide to prescription for field fertilization.

Materials and Methods

Topsoil (0-10 cm) was collected from site 2 on 14 December 1981, sieved to remove stones and organic particles > 0.5 cm in diameter, and thoroughly mixed. Field-moist soil equivalent to 750 g of air-dry soil was placed in plastic pots. Some characteristics of the soil are shown in Table C1.

The experiment was designed with eight treatments and four blocks. Within each block the pH of soil in the pots was adjusted (by the use of $CaCO_3$) to the values shown in Table C1. To determine the amount of $CaCO_3$ needed, quantities between 0 and 0.75 g were added to vials containing 10 g of soil and 20 ml of water and equilibrated with intermittent shaking for 18 hours. The neutralization curve obtained in this way is shown in Figure C1. $CaCO_3$ was mixed with the soil at the time of potting.

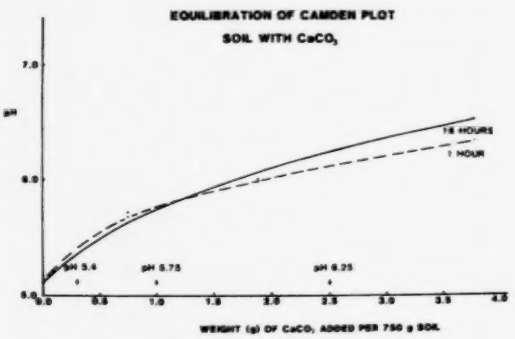


Figure C1. Equilibration of soil with calcium carbonate.

Table C1. Characteristics of soil used in pot experiment (four determinations)

pH (initial)	5.0 - 5.6
Total organic C (%)	5.8 - 6.0
Total N (%)	0.37 - 0.49
C/N	12.3 - 16.3
NH_4-N (ppm)	9 - 21
NO_3-N (ppm)	0.8 - 2.6
Extractable P (ppm)	1.3 - 2.3
CEC (me/100 g soil)	27 - 35
Base saturation (%)	18 - 23

	Adjusted pH		
Block 1 (control)	5.1	: 0.0 g $CaCO_3$	added per pot
Block 2	5.4	: 0.3 g	" "
Block 3	5.75	: 1.0 g	" "
Block 4	6.25	: 2.5 g	" "

	Treatments applied per block		
No.	ppm N	ppm P	ppm K
1	Nil	Nil	Nil
2	120(4N)	Nil	Nil
3	Nil	18	Nil
4	Nil	Nil	134
5	30 (1N)	18	134
6	60(2N)	18	134
7	90(3N)	18	134
8	120(4N)	18	134

N was applied as $(NH_4)_2SO_4$; P as H_3PO_4; K as KCl.

The eight treatments applied in each block are also shown in Table C1. Treatment 8 (120 ppm N, 18 ppm P and 134 ppm K) uses the elements in the ratio contained in Hoagland's solution.

Seedlings were grown in tubes of the test soil, without fertilization, from seed obtained from Roses Tier, just south of the experimental plot, and were transplanted one per pot on 23 December 1981 when they were 2-3 cm tall. After 20 days, the appropriate amount of fertilizer was added to each pot in 50 ml of water. Thereafter, when the seedlings began to wilt, the pots were set in 1 cm of water in trays overnight. Weeds were removed as they germinated, and the soil surface was cultivated at intervals to discourage growth of algae and moss.

The pots were placed in four trays, each tray containing one $CaCO_3$ treatment. The positions of treatments within trays, and of the trays themselves, were changed at random at intervals throughout the experiment.

Seedlings were measured for height at intervals of 2-3 weeks. They were harvested on 9 April, and leaves, shoots, and roots were analyzed for length (stem), weight, and area (leaves).

(b) N-mineralization Studies

Objective

To determine the effect of field and laboratory treatments upon the rate at which mineral N is supplied by the soil.

Materials and Methods

i. **Incubation experiments:** Soil was incubated with or without amendment by one or both of two methods.

a) Static incubation: A quantity of sieved, field-fresh soil from 0-10 cm depth equivalent to 10 g oven-dry weight was placed in a 250 ml Ehrlenmeyer flask, the top was sealed with Parafilm, and the flasks were placed in a constant-temperature room in the dark for 8 or 12 weeks. Field-fresh soil contained 30-50% water. When soil was amended with fertilizer, it was first dried to 20% moisture content and the fertilizer was added in solution to raise the moisture content to 40%. Details of each incubation experiment are given in Appendix 1.

b) Perfusion: Audus-type perfusion units were used (Audus 1946). Sieved, field-fresh soil from a depth of 0-10 cm, equivalent to 60 or 80 g oven-dry weight, was perfused with water or nutrient solution in a soil:perfusate ratio of 1:5 (W/V). Units were incubated at 20°C in the dark and the perfusate was sampled at weekly intervals. Perfusion was carried out for 8 hours a day from Monday to Friday, and the units were sampled each Friday. Details of the perfusion experiment are given in Appendix 1.

ii) **Chemical analyses:** After incubation, two subsamples of soil were shaken for 1 hr with 2N KCl, 1:5 soil:water (W/V) and filtered. (Subsamples were similarly extracted before incubation.)

In the perfusates and KCl filtrates, NH_4-N and NO_3-N were determined by micro-diffusion (Conway 1947, Bremner 1965), and NO_2-N was estimated colorimetrically by using the Griess-Ilosvay reagent.

Total organic C and total N were determined by the rapid method of Anstett (1958), and pH was measured with glass electrode in a 1:3 soil:water slurry (W/V).

Available P was extracted with dilute acid-fluoride (Olsen and Dean 1965) and was determined by the method of Murphy and Riley (1962). Cation-exchange capacity was determined by ammonium saturation as described by Chapman (1965), and exchangeable Ca, Mg, and K in the filtrate were determined by atomic absorption spectroscopy.

Results

(a) Seedling Pot Study

The growth responses of seedlings to the eight fertilizer treatments at four $CaCO_3$ levels are summarized in Table C2 and Figure C2.

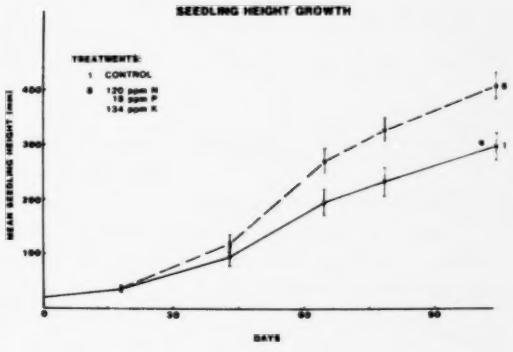


Figure C2. Effects of fertilization on **E. delegatensis** seedling height.

Calcium carbonate at 0.3 g per pot significantly increased the height, total weight, and mean weight per leaf of the seedlings, but at higher levels it only stimulated branching and leaf formation, and diminished root:shoot ratio. Hence, the principal effect of $CaCO_3$ was on the form of the seedling.

In most factors the greatest response was obtained with treatment 8 (4 NPK). In every measure, the response to 4 N was very similar to the response to 2 NPK. The root:shoot ratio diminished with increase in N; other responses increased with increase in N. Responses to P and K individually were small and non-significant.

(b) N-mineralization Studies

i. Static incubation: Experiment 1. In Tables C3 and C4, soil from the Camden Compt 49 plot is compared with that from six grass stands on Paradise Plains. These latter represent three pairs of plots: one pair near the rain forest edge at the eastern end of the Plains that has been under grass since destruction of the rain forest 50-100 years ago; one from the centre of the Plains that has been under grass probably for more than 100 years, but still carries isolated myrtle; and one from the western edge of the Plains in which only traces of old myrtle logs are occasionally found and grass has been present since well before settlement. The soil from Compartment 49 is similar in many respects to that from the centre of Paradise Plains and may be regarded as a reasonably typical grassland soil within the range presented here.

Experiment 2. The amounts of mineral N present in fresh soil varied during the growing season. At the start of the study, at the end of October 1981, the top 0-10 cm contained 11.3 ppm NH_4-N and 1.0 ppm NO_3-N. The values for control and

Table C2. A. Seedling response to NPK (Control = 100)

Measure of response	Treatment*							
	Control**	4N	P	K	1 NPK	2NPK	3NPK	4NPK
Height	100 (30.0 cm)^{a}	134^{d}	103^{a}	115^{b}	119^{bc}	131^{cd}	149^{e}	144de
Branch length	100 (6.5 cm)^{ab}	309^{abc}	140^{ab}	59^{a}	180^{abc}	332^{bc}	419^{c}	484^{c}
Total weight	100 (2.54 g)^{a}	167^{c}	113^{ab}	120^{ab}	141^{bc}	170^{c}	203de	228^{c}
Root:shoot	100 (0.216)^{b}	84^{ab}	91^{b}	93^{b}	97^{b}	86^{ab}	90^{b}	76^{a}
No. of leaves	100 (20.9)^{a}	126^{b}	95^{a}	93^{a}	103^{ab}	117^{bc}	128^{c}	131^{c}
Wt per leaf	100 (0.077 g)^{a}	125^{abc}	117^{ab}	123^{abc}	132^{bc}	140^{bc}	148^{c}	175^{d}

*1N = 30 ppm; 2N = 60 ppm; 3N = 90 ppm; 4N = 120 ppm; P = 18 ppm; K = 134 ppm.
**Mean values per seedling are given in parentheses for control only.

Table C2. B. Seedling response to $CaCO_3$ (Control = 100)

Measure of response	$CaCO_3$ (g) per pot			
	Control**	0.3	1.0	2.5
Height	100 (32.4 cm)^{b}	106^{b}	101^{b}	91^{a}
Branch length	100 (7.7 cm)^{a}	220^{a}	184^{a}	371^{b}
Total weight	100 (c.69g)^{a}	122^{b}	99^{a}	108^{ab}
Root:shoot	100 (0.233)^{c}	89^{b}	82^{b}	72^{a}
No. of leaves	100 (20.2)^{a}	120^{b}	108^{ab}	135^{c}
Wt per leaf	100 (0.114g)^{ab}	106^{b}	95^{ab}	90^{a}

**Mean values per seedling are given in parentheses for control only.
Data in columns with same letter are not significantly different at $P \leq 0.05$.

Table C3. Total organic C, N and pH of grassland soils

Location	pH	% Base saturation CEC*	Satur-ation	C%	N%	C/N
Camden						
1. Compt 49	5.6	30	20	6.2	0.43	15
Paradise Plains						
1. East	5.4	25	14	7.8	0.34	23
	5.3	26	17	8.6	0.34	25
2. Centre	5.3	23	21	7.6	0.40	19
	4.8	31	15	9.5	0.61	15
3. West	5.4	23	23	6.4	0.35	18
	5.1	27	16	6.0	0.32	19

[a] Millequivalents per 100 g oven-dry soil.

treated plots (fenced) on three dates during the summer are given in Table C5. Both NH_4-N and NO_3-N accumulated in treated soil during the summer with the greatest accumulation being in soil treated with herbicide. No nitrite was detected in either fresh or statically incubated soil.

Nitrification occurred during static incubation of soil from all three treatments. By late summer, significantly more NO_3-N was produced in soil from the herbicide and weeded treatments than from the control (Table C6). However, the net rate of production of mineral N ($NH_4^+ + NO_3^-$) was significantly lower in soil from the weeded treatment than in that from the herbicide treatment, which did not differ significantly from the control. From this it is concluded that short-term increase in the equilibrium levels of N in treated soil is due to lack of uptake by vegetation rather than to increased rate of production.

Experiment 3. Calcium carbonate alone did not stimulate nitrification or net mineralization of nitrogen (Table C7), but in samples treated with $(NH_4)_2SO_4$, $CaCO_3$ stimulated nitrification both alone and in combination with P. Phosphorus alone and in samples treated with $(NH_4)_2SO_4$ increased both the amount of nitrate and the total mineral N produced. Carbon, as glucose, caused immobilization of added NH_4-N and reduced net mineralization in unamended soil. The percentage of mineral N present as NO_3-N was not affected by the addition of glucose, and the relative effects of Ca and P were similar in soil treated with glucose and in untreated soil.

ii. Perfusion incubation: Experiment 1. When soils from the two single-tree experimental areas were perfused with distilled water in duplicate units, nitrification occurred after a short lag period and continued at low uniform rates of 0.96 and 0.68 µg N/g soil/day for sites 1 and 2, respectively (Fig. C3). The addition of 240 ppm NH_4-N increased this rate to 8.1 µg N/g soil/day in both soils between the 30th and 45th days after perfusion. After 56 days the two soils were mixed and replaced in four units, and perfusion was continued. By the 75th day the rate of nitrification was only 0.47 µg N/g soil/day and 80% of the NH_4-N added had appeared in the perfusate as NO_3-N. When a further 240 ppm NH_4-N were added to duplicate samples, nitrification proceeded without a lag period at a rate of 8.4 µg N/g soil/day. Concentrations of NO_2-N did not exceed 0.15 ppm in the perfusate at any time. Nitrification causes acidification of the soil and in the course of this experiment the pH of perfusates treated with $(NH_4)_2SO_4$ fell from 5.3 to 4.0. This change did not affect the rate of nitrification.

Experiment 2. Neither $CaCO_3$ nor P alone affected the rate of nitrification (Fig. C4, Table C7). In both these treatments and the untreated control, nitrification began after 1 week and continued for the next 12 weeks at a uniform rate of 1.2 µg N/g soil per day.

Table C4. Extractable P and mineral N in grassland soils collected March-April 1982 (ppm oven-dry soil).

Location	Oven-dry soil	Fresh soil			Net N after incubation[a] unamended			Net N after incubation[a] with 85 ppm NH_4-4		
	Extractable P	NH_4-4	NO_3-N	Both	NH_4-N	NO_3-N	Both	NH_4-N	NO_3-N	Both
Camden										
Compt 49	1.7	13.0	2.6	**14.6**	-5.4	32.3	**26.9**	-20.6	52.5	**32.0**
Paradise Plains										
1. East	0.7	21.7	1.3	**23.0**	-16.7	72.4	**55.7**	-69.5	135.9	**66.4**
	5.1	11.4	1.2	**12.6**	-9.9	23.2	**13.3**	-108.9	135.8	**26.9**
2. Centre	0.0	1.7	0.0	**1.7**	7.6	6.3	**13.9**	-1.1	16.9	**15.8**
	2.7	12.6	0.1	**12.7**	0.2	27.1	**26.9**	12.3	36.7	**49.0**
3. West	0.6	12.5	0.7	**13.2**	-10.4	5.9	**-5.5**	-19.2	22.1	**2.9**
	1.4	2.7	0.7	**3.4**	-1.2	-0.7	**-1.9**	-16.7	-0.7	**-17.4**

[a]Static incubation for 8 weeks.

Table C5. Mineral N in fresh soil (ppm) Camden Compt 49

Treatment	Dates sampled 12/2/82			11/3/82			20/04/82		
	NH_4-N	NO_3-N	Both	NH_4-N	NO_3-N	Both	NH_4-N	NO_3-N	Both
Control	4.9^{a}	0.0^{a}	4.9^{c}	11.8^{a}	1.1^{a}	12.9^{a}	14.2^{a}	2.0^{a}	16.2^{a}
Herbicide	17.8^{ab}	6.9^{b}	24.7^{b}	25.9^{b}	8.0^{ab}	33.9^{b}	45.4^{b}	19.6^{b}	65.0^{b}
Manually weeded	25.4^{b}	11.9^{b}	37.3^{b}	40.2^{b}	13.2^{b}	53.4^{c}	38.8^{b}	20.6^{b}	49.4^{b}

Within each column, figures with different superscript letters differ significantly (P = .05).

Table C6. Net mineral N produced during incubation (ppm) Camden Compt 49

	Dates sampled					
	11/03/82			20/04/82		
Treatment	NH_4-N	NO_3-N	Both	NH_4-N	NO_3-N	Both
Control	-4.2[a]	34.1[a]	**29.8[ab]**	-6.5[a]	30.4[a]	**23.9[ab]**
Herbicide	-21.2[b]	55.9[b]	**34.8[b]**	-28.8[b]	78.1[b]	**49.3[b]**
Manually weeded	-38.1	48.6[ab]	**10.5[a]**	-35.7	56.8[b]	**21.1[a]**

Within each column, figures with different superscript letters differ significantly (P = .05).

Table C7. Net mineral N (ppm) produced during incubation of amended soil for 13 weeks Camden Compt 49

	Static Incubation			Perfusion		
Treatment[a]	NH_4-N	NO_3-N	Total	NH_4-N	NO_3-N	Total
Control	-1.1	117.2	**116.1**	-11.5	99.8	**88.3**
C	-3.0	28.3	**25.3**	-11.5	2.6	**-8.9**
Ca	-7.1	117.2	**109.9**	-11.5	95.8	**84.3**
P	16.8	136.1	**152.9**	-11.5	98.6	**87.1**
N	-32.2	144.6	**112.4**	-228.5	296.5	**68.0**
N Ca	-100.2	212.6	**112.3**	-229.7	279.1	**49.4**
N P	-46.5	171.5	**125.0**	-228.9	287.4	**58.5**
N Ca P	105.6	226.8	**121.2**	-229.7	270.7	**41.0**
N C	-43.4	90.7	**47.3**	-186.6	87.8	**-98.8**
N C Ca	092.4	138.4	**45.9**	-210.2	119.1	**-91.1**
N C P	-73.8	124.7	**50.9**	-228.9	173.1	**-55.8**
N C Ca P	-108.3	147.4	**39.1**	-229.7	160.8	**-68.9**

[a]See Appendix for quantities used.

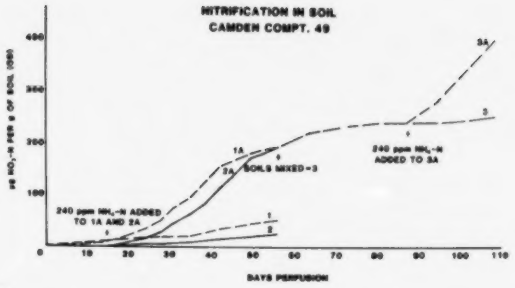


Figure C3. Nitrification in Camden soil.

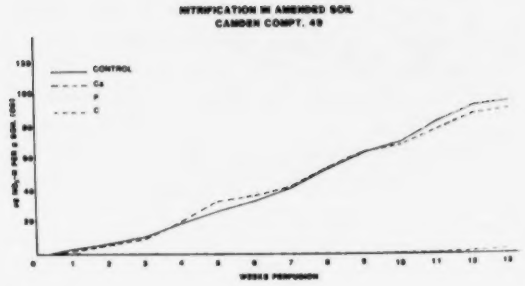


Figure C4. Nitrification in amended Camden soil.

The effect of P and Ca upon N-immobilization and nitrification with added NH_4-N is shown in Figures C5 and C6 and Table C7. About half of the N added as $(NH_4)_2SO_4$ was immobilized in the first 1-2 weeks before nitrification started, and this figure increased to 60% when Ca and/or P were added. Immobilization is probably effected by a combination of adsorption on the soil's exchange complex and microbial uptake that was enhanced, particularly by the addition of P (Fig. C5). After a lag period of 2-3 weeks, the amount of NH_4-N in the perfusate declined at 0.5 the rate of increase in NO_3-N, until about 80% of the added N had been nitrified (Fig. C6). Thereafter, the rate of increase of NO_3-N was the same for treated and control samples, despite high populations of nitrifiers in the former. Addition of either P or Ca increased the rate of nitrification and their effects were additive in combination. However, the total amount of NO_3-N produced after 13 weeks did not differ, appreciably, among treatments. Nitrite appeared between the third and tenth weeks in units treated with Ca or P, but concentration in the perfusate never exceeded 0.6 ppm.

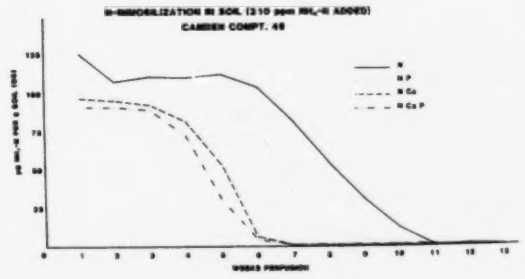


Figure C5. Nitrogen immobilization in soil amended with 210 ppm NH_4-N.

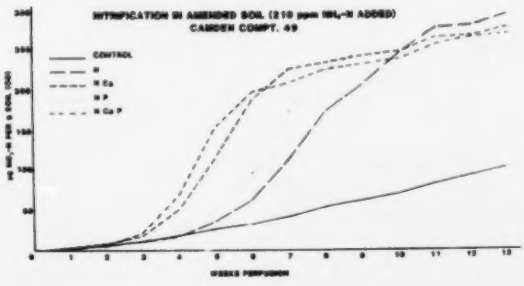


Figure C6. Nitrification in soil amended with 210 ppm NH_4-N.

Lees and Quastel (1946a) showed that, when soil is perfused with a solution of $(NH_4)_2SO_4$, the curve of nitrate accumulation against time has a sigmoid form similar to that encountered in bacterial proliferation studies: it could be represented by the equation:

$$\text{Log}\frac{y}{A-y} = k(t-t_1)$$

where y = NO_3-N produced (μg/g soil),
A = asymptotic value approached by y,
t = time from start of perfusion,
t_1 = time when y = 0.5A = time of half completion,
K = velocity constant.

In Figure C7 is shown the variation of Log y/A-y with time, together with the velocity constant, K, and the time to 0.5 completion of nitrification, t_1. Both Ca and P increased K, and shortened both t_1 and the initial lag period.

The effect of adding carbon as glucose is shown in Figure C8 and Table C7. With C+N, 87% of added NH_4-N was immobilized after 2 weeks; thereafter, about 10% was remobilized as soluble NH_4-N over the next 7 weeks. With the addition of either P or P.Ca to NC all added N was immobilized after one week. Over the next 6 weeks, 12-16% was remobilized as soluble NH_4-N. The addition of C depressed rates of nitrification for 6-8 weeks (doubling the lag period in comparison with the control experienced in the absence of added C). Thereafter, nitrification followed a pattern similar to that for equivalent treatments without C (Fig. C9), although the rates were decreased by about half. By the end of the experiment the percentage of added NH_4-N still immobilized ranged from 71% for NCP treatment to 111% for NC treatment, in comparison with 13 to 26% for treatments that did not include C.

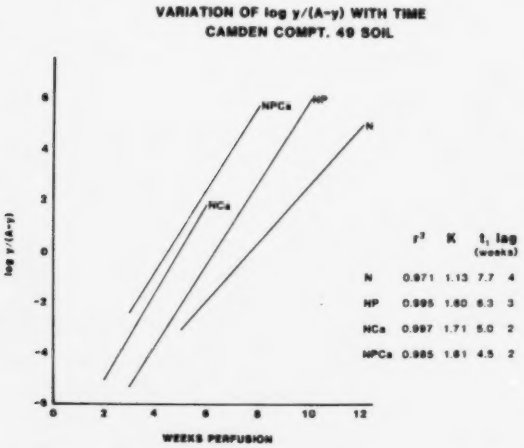


Figure C7. Velocity constants of nitrification in amended soil.

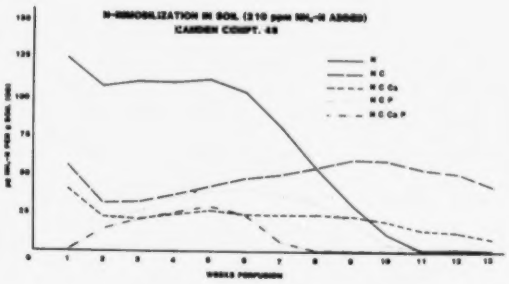


Figure C8. Nitrogen immobilization in soil amended with 210 ppm NH_4-N and glucose.

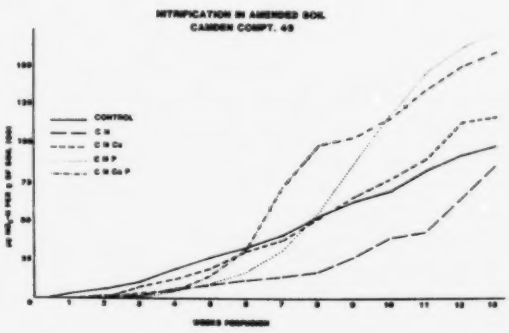


Figure C9. Nitrification in soil amended with 210 ppm NH_4-N and glucose.

Discussion

In all the characteristics measured, the soil used for these studies was reasonably typical of the grassland soils of the surrounding plateau. The content of C and N was high in comparison with that of some New Zealand tussock grassland, and the C:N ratio was relatively low. Exchangeable Ca, Mg, and K levels were as high as those in adjacent forest soils, but fewer than 2 ppm P were extracted by the acid ammonium fluoride method. In short, it was a relatively fertile grassland soil.

Seedlings grew fairly well in unamended soil in the pot-trial and did not respond significantly to P or K alone in any parameter of growth. However, there was a marked response to added N. In comparison with the control, there was a linear increase in mean total weight of seedlings with amount of N added, from 1 NPK through 4 NPK. The intercept of zero growth was at -85 ppm N. This could indicate that, in addition to the fertilizer, the soil supplied N at an average rate of 5.1 ppm per week during the 16.5 weeks of the experiment. In the three static and two perfusion incubation experiments using soil from or near the experimental plot, soil N was mineralized at an average rate of 6.3 ppm (3.5 to 9.8 ppm) per week for periods between 8 and 13 weeks.

Calcium carbonate appeared to increase seedling weight significantly when applied at a rate of 0.3g per pot (400 ppm); but at higher levels, its principal effect was to increase the branching and number of leaves, and to decrease root: shoot ratio progressively with an increase in the rate of application.

With the exception of a possible negative effect of K on branching, neither P nor K alone affected any of the parameters of growth significantly and both are probably non-limiting to growth in unamended soil. However, in total seedling weight, number of leaves, and weight per leaf, 4 NPK gave higher values than 4 N, and this suggests that there may be a significant PK or NPK interaction. During static incubation, it was found that P increased both the rate of nitrification and the total mineral N produced (Table C7), while K displaced exchangeable NH_4-N (Bremner 1965, Heilman 1975). Therefore, both P and K could increase the amount of N available to seedlings, and this may account for their positive effect in combination with N.

The equilibrium levels of NH_4-N and NO_3-N are fairly high for unimproved grassland (Table C4, C5) and were considerably enhanced by both herbicide and manual weeding treatment. Removal of grass and herbaceous vegetation by either treatment did not increase significantly the rate of mineralization of N during static incubation of soil samples collected after 6 and 7 months (Table C6), but did increase the proportion of mineral N converted to nitrate. This agrees with the observations of O'Connor et al. (1962) on 100-yr-old tussock grassland in New Zealand, although levels of mineral N there were generally much lower than those found here.

During perfusion with distilled water, nitrification in unamended soil began in the first 1-2 weeks and proceeded at a steady rate for the duration of each experiment. The rate was unaffected by the addition of either P or $CaCO_3$ sufficient to raise the pH of the perfusate by 0.5 units. This indicates that, contrary to the situation in grasslands studied by Robinson (1963) and Purchase (1974b), P was not deficient for normal nitrification in our soil, nor was CO_2 deficient (Barritt 1933).

When unamended soil was perfused with a solution of $(NH_4)_2SO_4$ the rate of nitrification followed the sigmoid curve, pre-

ceded by a lag period, as demonstrated by Lees and Quastel (1946), and Sabey et al. (1959). The rate of nitrification increased to more than 10 times that of the control, and this showed that a deficiency of ammonium substrate was the principal limitation to nitrification. Nevertheless, addition of either $CaCO_3$ or P to soil during perfusion with $(NH_4)_2SO_4$ shortened the lag period, increased the velocity constant of nitrification, K, and shortened the time, $t_{\frac{1}{2}}$ (the time required for half completion of the reaction) and the effects were additive (Fig. C7). The addition of $CaCO_3$ raised the pH by less than half a unit; hence, the effect of $CaCO_3$ was almost certainly expressed not through a change in pH but through an increase in available CO_2. Therefore, when the deficiency of substrate is removed, the limitation to rate of nitrification becomes a deficiency of CO_2 and P. These results agree with those of Richardson (1938) for grass leys rather than with those obtained from natural grassland (Robinson 1963).

None of the amendments increased the amount of soil N mineralized during perfusion, but, with static incubation, P in the absence of glucose did stimulate mineralization of soil N. A priming efect of added N on mineralization of soil N has been noted for some agricultural and forest soils (Broadbent 1965; Overrein 1967, 1970; Williams 1972; Johnson et al. 1980) but in our experiments it was not evident in either method of incubation.

During perfusion, about half of the added N was adsorbed almost immediately by the soil, and about one-fifth was permanently immobilized after 8-10 weeks. There was no appreciable immobilization of added N during static incubation. That immobilization of N was biological rather than chemical is shown by the change in the proportion of N immobilized when an energy source (glucose) was added (Fig. C8). After 4 days, 75% of the N was removed from solution in the CN treatment, 80% in the CNCa treatment, and 100% in the CNP and CNCaP treatments. The N was probably utilized by the heterotrophic soil organisms (stimulated by added C, as noted by Jones and Richards [1978]) for which P had then become a limiting element. The rate of remineralization and nitrification was greatest in the P treatments and least in the CN treatment (Fig. C9), and this reflected the stimulating effect of P upon biological activity.

Appendix 1: Incubation Methods

a) Static incubation

Experiment 1:

Soil (0-10 cm depth) collected from seven stands of grass from Paradise Plains and Pen Ridge, including that of the experimental plot in Compt 49, was sieved to remove material > 0.5 cm in size, and stored at 0°C for a maximum of 2 weeks. Four field-fresh samples of each soil, containing the equivalent of 10 g oven-dry soil, were placed in 250 ml Ehrlenmeyer flasks. A fifth sample was placed in an 80 ml vial for immediate extraction with 50 ml of 2N KCl (Bremner 1965). The pH was determined on a sixth sample of 25 g made into a paste with water in a weight: volume ratio of 1:2, and moisture content was determined on a seventh sample which was dried for 24 hr at 110°C.

All determinations were performed in duplicate. Samples for incubation were divided into two pairs per soil. To one pair was added distilled water to bring the moisture content to about 40% of moist soil weight, and to the other pair was added $(NH_4)_2SO_4$ solution to achieve an addition of 1 mg N per flask with a final moisture content of approx. 40% of moist soil weight. The tops were sealed with Parafilm.

Samples were incubated for 8 weeks in the dark at 20°C. This is approximately

the temperature of the soil at a depth of 10 cm on a hot summer day on the Camden Plateau. After incubation, the samples were extracted with 50 ml of 2N KCl, and NH_4-N and NO_3-4 were determined by micro-diffusion (Bremner 1965). Tests for the presence of NO_2-N were made with the Griess-Ilosvay reagent.

Experiment 2:

Soil was collected on two occasions from the three treatments of the experimental plot in Compt 49. Dates of collection were 11 March and 2 April, about 5 months after the treatments were applied. A composite sample from the 0-7.5 cm soil layer was taken from each replicate of the three treatments for a total of 27 samples on each date. Treatment and incubation of the samples were as for Experiment 1 above.

Experiment 3:

Composite samples from the surface soil layer 0-10 cm deep from around the plot were sieved and allowed to dry to a moisture content of 20%. As in Experiment 1, subsamples were taken for determination of pH, moisture content, and initial content of NH_4-N and NO_3-N. Twelve pairs of samples (10 g soil oven-dry equivalent) were weighed into 250 ml Ehrlenmeyer flasks and the following treatments were applied, one per pair of samples (expressed as ppm oven-dry soil).

1. Untreated control.
2. 1500 ppm C as glucose.
3. 250 ppm $CaCO_3$.
4. 50 ppm P as K_2HPO_4.
5. 100 ppm N as $(NH_4)_2SO_4$.
6. 100 ppm N + 1500 ppm C.
7. 100 ppm N + 2500 ppm $CaCO_3$.
8. 100 ppm N + 1500 ppm C + 2500 ppm $CaCO_3$.
9. 100 ppm N + 1500 ppm C + 2500 ppm $CaCO_3$ + 50 ppm P.
10. 100 ppm N + 1500 ppm C + 50 ppm P.
11. 100 ppm N + 2500 ppm $CaCO_3$ + 50 ppm P.
12. 100 ppm N.

The nutrients were added in solution and the moisture content of the soil was adjusted to about 40% of moist weight. The tops of the flasks were sealed with Parafilm.

Incubation was continued for 12 weeks, and the samples were then extracted with 50 ml 2N KCl.

b) Perfusion incubation

Experiment 1:

On 29 October 1982, soil (0-10 cm depth) was collected from each of the two areas in Compt 49 selected for the single tree studies. Two bulked samples of 20 cores were taken from each area, designated 1A/1B; 2A/2B. Fresh soil from each sample, equivalent to 68g oven-dry weight, was put into duplicate Audus-type units (Audus 1946) and perfused with 250 ml of distilled water. This gave a total water content of about 285 ml per unit. After 15 days' perfusion $(NH_4)_2SO_4$ was added to four of the eight units at a rate of 240 µgN/g oven-dry soil and $CaCO_3$ was added to two units at a rate of 5.6 mg $CaCO_3$/g oven-dry soil.

Units were sampled at intervals of 3 and 4 days, 2 ml being taken for analysis, and perfusion was continued from 8:30 a.m. to 5:00 p.m. Monday to Friday inclusive.

After 8 weeks all units were drained. Subsamples from each unit were extracted with 2 N KCl, and exchangeable (extractable) NH_4-N and NO_3-N were determined. Soil from the four units treated

with $(NH_4)_2SO_4$ was then thoroughly mixed, the equivalent of 68 g oven-dry weight of the mixture was put into each of three units and 250 ml of distilled water were added. Perfusion was continued for four weeks and each week the perfusate was drained and replaced with distilled water. On the 87th day, two units were treated with $(NH_4)_2SO_4$ as before and perfusion continued for a further 3 weeks.

At the end of the experiment, subsamples of soil from each unit were extracted with 2N KCl for the determination of exchangeable NH_4-N and NO_3-N. In calculating the quantities and rates of transformation of N, allowance was made for evaporation from the units and for the quantities of perfusate removed for analysis. With the quantity of soil used, all duplicate samples agreed very closely. Therefore, in subsequent experiments single units of each treatment were used.

Experiment 2:

On 7 May 1982 soil was collected from around the fenced plot in Compt 49. The soil (from 0-10 cm depth) was sieved and subsamples were extracted for determination of initial exchangeable NH_4-N and NO_3-N. Further subsamples were used for determination of pH and moisture content.

Fresh soil equivalent to 80 g oven-dry weight was put into each of 12 perfusion units. The following fertilizer treatments were applied to the units (expressed as ppm in perfusate).

Unit	Amendment
1.	Untreated control.
2.	750 ppm as glucose.
3.	$CaCO_3$, 585 ppm.
4.	P, 25 ppm as KH_2PO_4.
5.	N, 50 ppm as $(NH_4)_2SO_4$
6.	N, 50 ppm; C, 750 ppm.
7.	N, 50 ppm; $CaCO_3$, 585 ppm.
8.	N, 50 ppm; C, 750 ppm; $CaCO_3$, 585 ppm.
9.	N, 50 ppm; C, 750 ppm; $CaCO_3$, 585 ppm; P, 25 ppm.
10.	N, 50 ppm; C, 750 ppm; P, 25 ppm.
11.	N, 50 ppm; $CaCO_3$, 585 ppm; P, 25 ppm.
12.	N, 50 ppm; P, 25 ppm.

Fertilizer was added as solution; the total amount of water in each unit was 342 ml, including original soil moisture.

Perfusion was carried out from 8:30 a.m. to 5:00 p.m., Monday to Friday inclusive. Each Friday a sample of perfusate (3 ml) was taken for determination of pH, NH_4-N, and NO_3-N, and for estimation of NO_2-N. The experiment was continued for 12 weeks.

SECTION D: WATER RELATIONS MEASUREMENTS

a) Calibration of instruments

i) Diffusion porometer

Stomatal resistance measurements were made with a Licor diffusion porometer fitted with a LI20S sensor as described by Kanemasu et al. (1969). The sensor was calibrated over a stainless steel plate with holes of known resistance to water vapor diffusion from a filter paper wick placed beneath the plate, at temperatures of 4, 11, 22, 24 and 30°C. Correction factors (C.F.) to convert data to base 24°C were calculated. The relationship with temperature is described by the equation: ln (C.F.) = 0.0723 T (°C) - 1.7328 (r = 0.99). The measured time (t sec) taken for relative humidity change between 16 and 24% at a particular temperature was multiplied by the C.F. to convert to base 24°C and was used in the following equation to obtain diffusive resistance (R) at 24°C.

R ($sec \cdot cm^{-1}$) = 2.2314 t (sec. converted to 24°C) - 7.5778

(r = 0.99)

ii) Dew point thermocouple psychrometer

A Wescor HR33T dew point microvoltmeter fitted with a C-52 sample chamber was used to measure leaf osmotic potential by the dew point method according to Brown (1971). All measurements were made on expressed sap from frozen (-35°C liquid freon) and thawed leaf discs. Sap was absorbed into filter paper discs which were then placed in the sample chamber. Samples were allowed to equilibrate for 5 minutes in the sample chamber prior to measurement. The instrument was calibrated with filter paper discs soaked in standard solutions of sodium chloride varying over a range of osmotic potentials from 0.05 Molal (-2.34 bars, -234 K Pa) to 1.0 Molal (-46.40 bars, -4640 K Pa). Osmotic potentials of expressed sap were obtained from the following equation.

Y (-Bars) = 1.540 x (μv) - 0.546
(1 Bar = 100 K Pa) r = 0.99

iii) Pressure chamber

Leaf water potentials were obtained from shoot xylem pressure potential determinations on excised shoots with a pressure chamber (Soil Moisture Ltd.) according to Ritchie and Hinckley (1975). In the field a 10-15 cm branch with several leaves on it was excised. Leaf diffusive resistance was measured with the porometer, and the branch was placed immediately in the pressure chamber. The pressure in the chamber was increased slowly until moisture began to appear at the cut end of the stem. This pressure was recorded and was used as a measure of leaf water potential. Leaf discs were punched from the same excised twig, were frozen in sealed vials with liquid freon, and were stored at -20°C for osmotic potential determinations.

iv) Solarimeter

Solar radiation was obtained at the time of measurement of water relations parameters in the field from a Kipp and Zonen solarimeter (type CM5) placed in an unobstructed exposed position. The manufacturer's calibration of the instrument (1 $W.cm^{-2}$ equals 117 mV) was used to convert mV readings to light energy units.

v) Relative humidity

Relative humidity was measured with an Asman-type psychrometer and absolute humidity deficits were calculated accord-

ing to tables 4.8-2 and 4.8-3 of the International Meteorological Tables (1966).

b) Experiment 1: Response of E. delegatensis Seedlings to Varying Soil Moisture Levels

Seedlings of **E. delegatensis** 20-30 cm high with two major lateral branches and eight leaves on the main stem were used in the experiment. The plants were grown from seed (Waddamana Seed Source) in the glasshouse and were actively growing at time of removal. Watering was withheld and six pots were allowed to reach different levels of moisture content including one that caused visible signs of wilting in seedlings. Seedlings were equilibrated for approximately 2 days under laboratory conditions of air temperature 24°C, relative humidity 56%, corresponding to an absolute humidity deficit of 9.5832 $\mu g \cdot cm^{-3}$ with lighting consisting of a mixture of diffused sunlight and incandescent light. During the mid-afternoon, the following parameters were measured: Stomatal resistance of one side of a fully expanded leaf (previous work having indicated similar stomatal resistance on abaxial and adaxial leaf surfaces), leaf water potential and leaf osmotic potential as outlined above. Turgor pressure was calculated by subtracting osmotic potential from leaf water potential. Soil moisture content was measured gravimetrically and converted to soil water potential by means of a matric suction soil moisture curve determined for that soil.

Results and Discussion

Leaf water potentials of up to -18.6 bars (-1860 K Pa) were induced in the treatments (Table D1). Visible wilting occurred between -8.3 and -13.2 bars, corresponding to the change in turgor of +3.5 bars to -3.9 bars, respectively, and an osmotic pressure of approximately -12 bars. On these plants, in contrast to older plants, leaf tissue was not heavily sclerophyllous and wilting was easily observed. Leaf diffusive resistance was markedly dependent on leaf water potential, reaching over 35 $sec \cdot cm^{-1}$ prior to the leaf undergoing negative turgor (Fig. D1). It would appear that stomatal closure occurred between -7.6 and -8.3 bars leaf water potential (using 30 $sec \cdot cm^{-1}$ and above as stomatal closure [Gee and Federer 1972]).

Table D1. Water relations parameters at varying soil moisture levels.

Soil (bars)	Leaf (bars)	π (bars)	Turgor (bars)	Leaf diffusive resistance R($sec \cdot cm^{-1}$)
- 3.6	- 5.8	-10.5	+ 4.7	10.11
- 4.7	- 7.6	- 9.1	+ 1.5	14.50
- 3.6	- 8.3	-11.8	+ 3.5	35.24
- 5.0	-13.2	- 9.3	- 3.9	70.79
- 4.8	-18.0	-14.2	- 3.8	83.64
- 7.2	-18.6	-16.8	- 1.8	142.12

(1 bar = 100 K Pa)

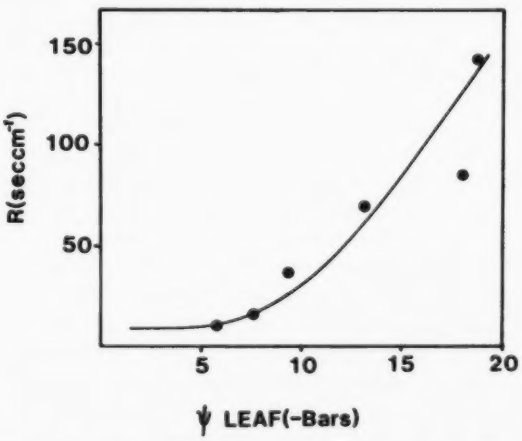


Figure D1. Relationship between leaf diffusive resistance and leaf water potential.

Stomatal resistance continued to increase up to 142 $sec \cdot cm^{-1}$, and this corresponded to leaf water potential of -18.6 bars.

It would appear that conditioning can alter the level of leaf water potential critical for stomatal closure since field conditioned seedlings on the Camden field site were able to undergo leaf water potentials of -14.8 bars without stomatal closure. It would also appear, however, that growth ceased since these seedlings were under negative turgor of -2.3 bars. A poor correlation was obtained between soil water potential and leaf water potential (Table D1), possibly as a result of the confined root system and the limited soil volume in the pot. Obviously, pot studies of seedling water relations have major limitations of which altered root growth and function in the constraining pot are of prime importance.

c) Experiment 2: Estimation of Transpiration Rate from Stomatal Resistance

Seedlings of E. **delegatensis** similar to those used in Experiment 1 were placed under laboratory environmental conditions. Plants were well watered and allowed to drain; the pot was then enclosed in plastic which was sealed around the stem. Gravimetric water loss measured every 15 min on three seedlings decreased over the first 45 min to a steady level. Transpiration data obtained after this initial equilibration period were used for comparison with an estimated transpiration rate calculated from stomatal resistance and absolute humidity deficits.

Stomatal resistance was measured on one side of a fully expanded leaf with a diffusion porometer, and leaf areas were calculated by photocopying leaves and weighing them as described in Section A. Transpiration was then calculated per seedling as absolute humidity deficits ($\mu g \cdot cm^{-3}$) divided by stomatal resistance ($sec \cdot cm^{-3}$) and multiplied by seedling leaf area. Boundary layer resistance was ignored in this calculation.

Results and Discussion

The calculated transpiration rate was similar to actual rates over the relatively short 45 min measurement period (Table D2). Calculated values underestimated actual rates by approximately 25-33%. This deviation could well result from the possible existence of different stomatal resistances of adaxial and abaxial surfaces of these young leaves (Pereira and Kozlowski 1976). Small differences between stomatal resistances of abaxial and adaxial surfaces of mature sclerophyllous leaf tissue were noted in our field experiments described in Section A; consequently, our initial assumption that this condition also applied to younger leaves may be invalid, and requires further testing.

Table D2. Seedling transpiration.

	Transpiration (g H_2O)	
Seedling No.	Calculated	Measured
1	0.08	0.12
2	0.12	0.16
3	0.09	0.11

SECTION E: EFFECTS OF FROST ON E. **DELEGATENSIS** SEEDLINGS

a) Frost tolerance

The high-altitude areas of north-eastern Tasmania offer a severe climate for eucalypt regeneration (Table E1). These areas are highly susceptible to unseasonable frosts and when frosts occur during active vegetative growth they can have a marked effect on seedling growth and form. Little is known at present about the frost resistance of **E. delegatensis** and hence it is difficult if not impossible to relate recorded microclimate data to seedling response. It is necessary to know, for example, minimum temperatures for seedling survival and minimum temperatures for damage to various seedling parts. Differences in frost hardiness undoubtedly occur between naked buds, leaves and stem tissue. The damage to seedlings from frost is a probable contributor to seedling growth check. Loss of naked buds results in increased branching and loss of apical dominance. Relatively minor frost damage occurring over prolonged periods with progressive reduction in leaf area can lead to a gradual decline in the competitive ability of the plant and, eventually, to cessation of growth and seedling mortality.

Many of the current methods for assessing frost hardiness are time consuming and costly, requiring elaborate facilities for precise temperature control (Levitt 1972, Paton 1972, Warrington and Rook 1980). Similarly, assessment procedures are time-consuming and in many cases subjective (Levitt 1972). However, procedures are available for quantitative estimation of frost damage (Timmis 1976, Warrington and Rook 1980) to which the electroconductivity diffusate method of Dexter (1932) is readily adaptable. Modifications of the method have been used on a wide range of plant species, e.g., Scots pine (**Pinus silvestris** L.) (Aronsson and Eliasson 1972), radiata pine (**P. radiata** D. Don) (Green and Warrington 1978), white spruce (**Picea glauca** [Moench] Voss), black spruce (**P. mariana** [Mill.] B.S.P.), jack pine (**Pinus banksiana** Lamb.) (Colombo et al. 1982), Douglas-fir (**Pseudotsuga menziesii** [Mirb.] Franco)(van den Driessche 1969) and apple (**Malus** sp.) (Wilner 1960) for assessing damage after artificial frosting of plant parts. Exposure of tissue to low, damaging temperatures results in increased cell membrane permeability with a concomitant increase in leakage of electrolytes that can be quantitatively estimated by measuring the conductivity of the bathing solution. In most species examined (Levitt 1972) conductivity is correlated to tissue damage. Because of the ease and rapidity of measurement and relevance to frost damage (Levitt 1972) the method was examined for frost hardiness determinations on E. **delegatensis.**

Materials and Methods

The method of Colombo et al. (1982) was employed with minor modification to suit sampling of broad-leafed tissue. Seedling leaf, stem or bud tissue was selected from individual plants, subsampled and randomized between 13 treatments consisting of 12 temperature treatments (-1°C to -12°C) at 1° intervals and a control treatment consisting of storage over ice. For leaf tissue, approximately six fully expanded leaves were removed from each of three to six plants per treatment and subsampled by punching equal numbers of 8 mm discs from the leaves. The leaf discs (7-10 per treatment) were randomized between treatments. Leaf discs were placed on paper tissue dampened with distilled H_2O and surrounded by aluminum foil. A 26-gauge thermocouple was placed

Table E1a. Rossarden meteorological data (monthly means)

	J	F	M	A	M	J	J	A	S	O	N	D	Year
Rainfall (mm)	54	66	62	80	87	82	93	102	85	90	73	79	953
Max temp (°C)	20.7	20.8	18.7	15.2	11.7	9.1	0.9	9.4	11.4	14.7	16.0	17.9	14.6
Min temp (°C)	8.1	9.0	7.2	5.1	2.7	0.6	0.2	0.9	2.2	2.9	5.4	6.8	4.3
Frost frequency (days/month)	0	0.1	0.6	2.1	7.1	12.3	14.0	10.4	6.1	4.6	0.9	0.4	58.6

Table E1b. Rainfall distribution at Upper Blessington

	J	F	M	A	M	J	J	A	S	O	N	D	Year
Rain (mm)	50	63	54	84	102	114	137	127	98	95	66	68	1358

on the discs and inside the foil to monitor leaf temperature at 1- and 5-min intervals as required, and the data were fed into a Doric "Digitrend 220" data logger. In some cases, where randomization of samples was not necessary, whole leaves were used for frosting tests. Leaf discs were then obtained after frosting to facilitate conductivity measurements. The foil-plus-thermocouple was placed inside a wide-mouth thermos flask and stoppered with a 3.5-cm-thick styrofoam bung. The flask was equilibrated at room temperature (approximately 18-20°C) and then placed in a commercial freezing chest at a temperature of -10° to -18°C depending on the rate of decrease in temperature desired. When the leaf reached the desired temperature, the flask was removed and allowed to warm at room temperature. Rate of decrease in temperature below 0°C was approximately 3-5°C/hr, with similar warming rates. When leaf temperature had reached +5.0°C the flask was opened and the leaf discs were placed in stoppered glass vials containing 10 ml of distilled H_2O. The vials were incubated at 20°C for 18 hr, after which they were thoroughly mixed (including the leaf discs) on a vortex mixer and the conductivity of the bathing solution was measured with a Philips conductivity bridge, model GM 4249/01 fitted with a GM 4221 measuring cell. In some cases vials were placed in a boiling water bath for 7 minutes to kill the tissue. They were allowed to incubate a further 18 hr at 20°C, after which conductivity of the bathing solution was remeasured. Dry weights of measured tissue were determined after drying at 65°C for 24 hr.

Data were expressed as conductivity per gram dry weight of tissue, percentage difference from control, relative conductivity (Colombo et al. 1982), or index of injury (Flint et al. 1967, Green and Warrington 1978).

Results and Discussion

Use of large, wide-mouth vacuum flasks (705 refills) stoppered with styrofoam bungs reduced the temperature variation of the freezing chest from a maximum of ± 2.0°C over a 20-min cycle time to less than ± 0.3°C at a mean temperature of -11.0°C. The buffering capacity of the flasks allowed for a temperature decrease and increase rate (3-5°C/hr) within the normally accepted range for artificial freezing tests (Levitt 1972). By altering the freezer temperature, rates of decrease of 1-12°C/hr^{-1} could be achieved.

Exposure of E. **delegatensis** unhardened leaf tissue to damaging temperatures resulted in a marked visible response. Leaf color changed from the healthy bright green of the unfrosted controls to a dark silver grey over a period of 7-10 days of incubation in distilled water at room temperature. No gradation in frost damage could be assessed visually.

A similar response was observed in longitudinally split 2.5-cm stem sections incubated at room temperature in distilled water. During the 7- to 10-day incubation period, frosted stems showed increasing brown coloration in the cambial region, with a gradual loosening of the bark. A similar response has been observed in apple stem section (Quamme et al. 1973), with the amount of oxidative browning being used to assess degree of frost damage. The lack of a graded visible response to frosting treatments did not allow for the testing of a quantitative relationship between visible damage and conductivity of the diffusate as was done in previous work on northern temperate forest species (Levitt 1972). However, it was observed over a wide range of frosting tests that visible damage was always associated with conductivity values (differences from controls) of over 100% (Fig. E1). For unhardened leaf tissue of E. **delegatensis** (Waddamana seed source) damage occurred at approximately -6.0°C and for unhardened stem tissue at -8.0°C. Exposure of these tissues to lower temperatures resulted in increased conductivity values, which suggests increased damage of cellular membranes associated with increased permeability and loss of electrolytes. Conductivity per gram dry weight of frosted tissue percentage difference from unfrosted controls was chosen over other units of measure (Materials and Methods) to express frost damage. Comparisons between different tissue types were possible with the ease and rapidity of calculation. In general, leaf tissue showed a greater increase in conductivity of the diffusate in response to exposure

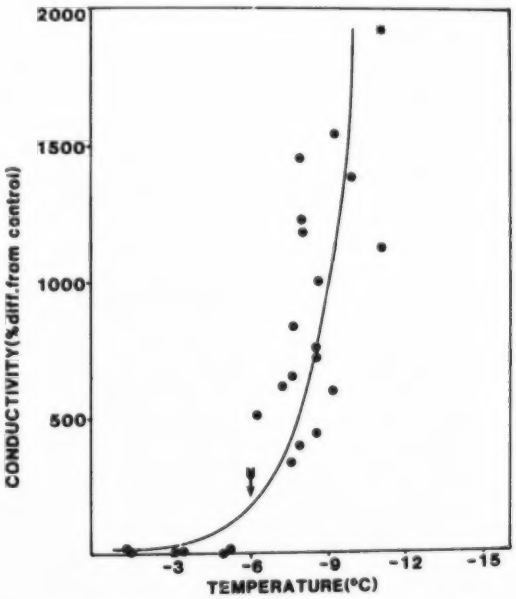


Figure E1. Effects of temperature on electrolyte leakage from unhardened E. **delegatensis** leaf tissue. Arrow indicates temperature at which visible damage occurred.

to damaging temperatures than did stem tissue per unit dry weight. This may reflect a higher mobility and/or quantity of inorganic ions, sugars, amino acids, etc., in leaf tissue (Levitt 1972). The sensitivity of leaf tissue to damaging temperatures suggests that it is suitable for use in assessing differences in frost hardiness.

The lack of a graded visible response to freezing temperatures of E. **delegatensis** leaf and stem tissue suggests that some critical event or process determines whether or not the tissue will be damaged. A similar conclusion has been reached by Paton (1981). In many species that do not develop tolerance to low temperatures the formation of ice crystals in the tissue results in critical damage (Burke et al. 1976) and, therefore, the ability of these tissues to supercool even a few degrees before ice formation occurs is a decided advantage. In E. **delegatensis,** the point at which ice formation occurs in the tissue can be detected from the rise in temperature or exotherm produced when water freezes as a result of the heat of crystalization (Fig. E2). In initial tests, leaf tissue (unhardened Waddamana seed source) was placed in aluminum foil together with a thermocouple and subjected to the normal temperature decrease rates outlined previously. Flasks were removed from the freezer when leaf tissue reached the desired temperatures and were assessed for visible damage. The presence of an exotherm indicated tissue damage (Fig. E2). Tissue that did not freeze and hence showed no exotherm was not damaged. Thus it appeared that unhardened E. **delegatensis** leaf tissue could not tolerate freezing and ice formation. A further test was undertaken to examine the presence of exotherms and the degree of leaf damage. Unhardened E. **delegatensis** leaves from Waddamana seed source growing in the greenhouse were exposed to a range of freezing temperatures which resulted in tissue freezing in some samples and supercooling without tissue freezing in others (Table E2). Damage was assessed by the conductivity method. In all replications that indicated tissue freezing, i.e., in those which showed an exotherm, tissue damage was the result. However, tissue damage that did not indicate tissue freezing was also found in other replications (No. 6 and 11, Table E2). In general in unhardened tissue, the presence of an exotherm indicating tissue freezing also resulted in frost damage. However, it would appear that damage can also result without the formation of ice in the tissue.

A further test was undertaken to examine the response of E. **delegatensis**

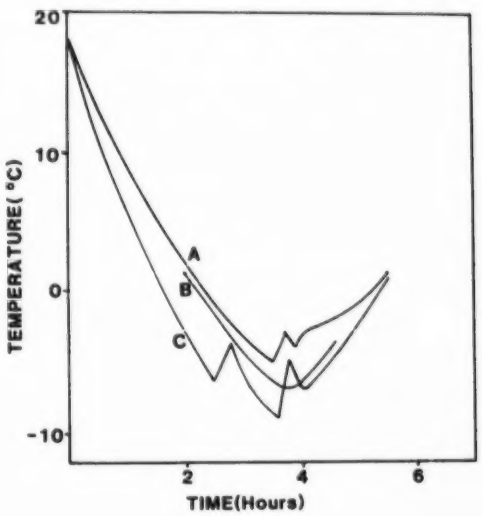


Figure E2. Temperature profiles for unhardened E. **delegatensis** leaf tissue. Samples A, B and C contained two leaves. No damage occurred in B, one leaf was damaged in A and both were damaged in C.

leaf tissue to freezing after hardening to cold temperatures. E. **delegatensis** seedlings (Waddamana seed source) grown outdoors and exposed to natural hardening through May and June were used. Two treatments were employed. Leaves were exposed to freezing temperatures as before (leaf dry), and exposed to freezing temperatures but with paper tissue dampened with distilled water surrounding the leaf to avoid marked supercooling (leaf wet). Flasks were removed before and after initiation of the exotherm, and tissue damage was tested by the conductivity method. Low temperature exotherms in the "leaf dry" treatment (-10.8, -9.2) resulted in tissue damage as did exposure to -10.8°C without freezing, although the amount of damage was reduced (Table E3).

The "leaf wet" treatment showed higher temperature exotherms (-2.7 to -4.1°C), but did not show damage unless exposed to a lower temperature of -9.2°C. In this instance, tissue freezing at -2.9°C and exposure to -9.2°C resulted in similar damage to treatments that supercooled to -9.2° and -10.8°C before freezing. It would appear that, in hardened leaf tissue, ice formation can be tolerated without damage so long as the temperature does not drop below a critical level, in this case -9.2°C.

In the unhardened leaf tissue, the ability to supercool can have a marked effect on the temperature that can be tolerated, i.e., it acts as a frost avoidance mechanism (Thomas and Barber 1974); however, it is no guarantee against damage when temperatures become low enough, even though ice formation does not take place. Nevertheless, in the hardened state there

Table E2. Unhardened E. **delegatensis** leaf tissue

Replication No.	Temp of exotherm initiation (°C)	Lowest temp reached (°C)	Conductivity % diff. from control
1		- 6.8	6
2	- 7.0	- 8.3	882
3	- 6.8	- 6.8	602
4	- 8.9	- 9.1	640
5	- 7.1	- 8.0	449
6		- 7.8	256
7		- 8.7	25
8	- 4.5	- 8.5	489
9	- 7.6	-10.0	522
10	- 8.5	- 8.5	1141
11		- 7.8	541
12		- 7.7	0

Table E3. Hardened E. **delegatensis** leaf tissue

Treatment	Rep.	Temp of exotherm initiation (°C)	Lowest temp reached (°C)	Conductivity % diff. from control
Leaf dry	1	-10.8	-10.8	411
	2	- 9.2	- 9.2	407
	3		-10.8	296
	4		- 8.4	0
	5		- 8.8	0
	6		- 8.0	0
Leaf wet	1	- 3.7	- 3.7	0
	2	- 2.9	- 9.2	411
	3	- 4.1	- 4.1	0
	4	- 2.7	- 2.7	0
	5		- 3.8	0
	6		- 4.1	0

appears to be no advantage to supercooling as formation of ice in leaf tissue can be tolerated without damage at relatively warm (-3°C to -4°C) temperatures. It is difficult to determine, once ice formation has occurred, whether damage results from exposure to a more severe low temperature or to the length of time the tissue is exposed to internal ice. Obviously, length of exposure is important in the degree of damage obtained (Levitt 1972), but in this test the increased period of exposure in the "leaf wet replication no. 2" treatment was negligible in comparison with that in "rep. 3". This is based on the observation that once the tissue freezes the rate of further cooling is increased.

In most methods of estimating frost tolerance, inoculation of plant tissue with ice crystals or wrapping in wet paper tissue is necessary to ensure freezing (Levitt 1972) and avoid deep supercooling. A comparison of snow inoculation and use of wet paper tissue surrounding the leaf indicated that the methods were equally effective in inducing ice formation (Table E4) at temperatures well above those necessary for ice formation in the dry leaf which showed marked supercooling without inoculation. In all subsequent tests, plant tissue was wrapped in paper tissue moistened with distilled water to induce ice formation.

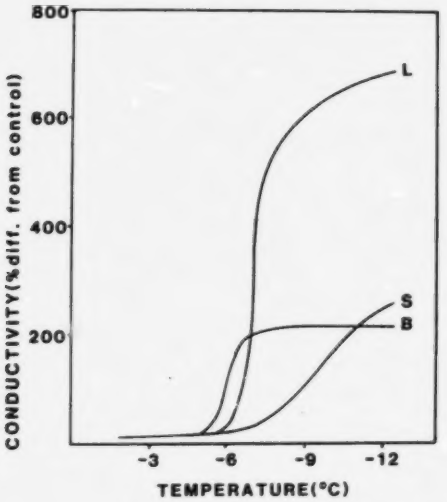
As shown previously, differences in frost tolerance by leaves and stems were observed. A further comparison was obtained with the Bicheno provenance of **E. delegatensis.** Samples of leaf, stem and bud tissue were obtained on 10 June 1982 from 3-year-old trees grown outdoors at Tarraleah. They were subjected to frost tolerance testing as previously described and were assessed by the conductivity method. The apical shoot, including the naked buds, was the least frost tolerant: damage was observed at a temperature of -6°C (Fig. E3). Leaf tissue showed damage at -7°C, but stem tissue did not show damage until -9°C. As noted previously, the increased response of the leaf tissue to damaging temperatures makes it a suitable tissue for comparative testing for frost tolerance of experimental treatments, provenances, etc., and it was therefore adapted for use in further testing.

Table E4. Effects of various inoculation procedures on freezing of leaf tissue.

Treatment	Rep.	Temp of exotherm initiation (°C)
Dry leaf	1	-13.6
	2	- 9.0
	3	-17.5
Wet leaf	1	- 4.7
	2	- 4.2
	3	- 4.5
Dry leaf+	1	- 5.5
Snow inoculation	2	- 4.3
	3	- 5.5

b) Provenance variation in frost tolerance of E. delegatensis

In October, 1979 a provenance trial of **E. delegatensis** was established in cooperation with the CSIRO Division of Forest Research and Australian Newsprint Mills at Tarraleah (latitude 42°20'S, longitude 146°25'E) which consisted of 69 individual provenances covering the range of **E. delegatensis** in Australia. Five provenances were selected in June, 1982 which ranged in degree of frost tolerance

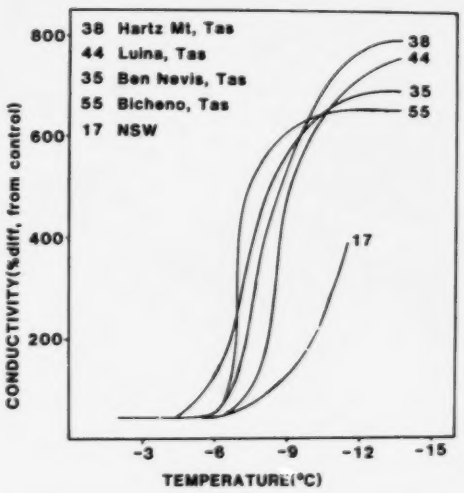


FROST HARDINESS OF LEAVES, STEMS AND BUDS OF E.delegatensis (Bicheno provenance)

Figure E3. Effects of temperature on electrolyte leakage from leaves (L), stems (S) and buds (B) of E. **delegatensis** trees of the Bicheno provenance.

(assessed after a natural frost in the Bago State forest provenance field trial[1]) from the least hardy (Bicheno, Tas. No. 55) to the most hardy (Gungarlin River, N.S.W. No. 17) (Table E5). In June, 1982 after natural hardening had occurred, approximately six fully expanded leaves from each of three to six trees per provenance were sampled. Leaves were stored over ice in a cool chest and transported to Hobart. The following day the leaves were subsampled (discs 8 mm in diameter punched from the leaves) and randomly distributed among 13 treatments as outlined in the MATERIALS AND METHODS section. After exposure to temperatures varying from -1°C to -12°C the leaf discs were warmed to room temperature and frost damage was assessed by the conductivity method. Exposure of leaves from the range of provenances sampled showed differences in degree of damage (Fig. E4). Summation of conductivity values over the critical temperature range in which damage occurs, i.e., -5.0°C to -9.0°C in 1°C intervals, allowed for a frost tolerance rating to be applied (Table E6). Increased conductivity above control values indicates increased damage. On this basis the frost tolerance ratings of the provenances tested in increasing degrees of damage were:

[1]D.J. Boland, CSIRO Division of Forest Research, Canberra, A.C.T. pers. comm.



FROST HARDINESS OF SOME E.delegatensis PROVENANCES

Figure E4. Effects of temperature on electrolyte leakage from leaf tissue of E. **delegatensis** trees from various provenances.

Table E5. E. **delegatensis** provenances

Provenance	Locality	Lat (S)	Long (E)	Elevation (m)
#17	Gungarlin R., N.S.W.	36°17'	148°30'	1210 - 1300
#35	Hartz Mt., Tas.	43°13'	146°52'	560
#38	Ben Nevis, Tas.	41°22'	147°40'	820
#44	Luina, Tas.	41°28'	145°22'	450
#55	Bicheno, Tas.	41°53'	148°12'	460

(1) No. 17 N.S.W.
(2) No. 44 Luina, Tas.
(3) No. 38 Ben Nevis, Tas.
(4) No. 35 Hartz Mt., Tas.
(5) No. 55 Bicheno, Tas.

These data agree closely with those obtained by Boland (pers. comm.) in field assessments of frost damage of these provenances after natural frosts in the Bago State Forest (N.S.W.).

Table E6. Frost hardiness of E. **delegatensis**

Provenance	Conductivity[a] rating	Frost hardiness rating
17	340	1
55	1670	5
35	1610	4
38	1260	3
44	630	2

[a]Conductivity data read from curve (Fig. E4) and summed over temperature range -3 to -9 at 1°C intervals.

Temp. at which first damage (>100%) occurs

17	-8 to -9
44	-7 to -8
38	-6 to -7
55	-6 to -7
35	-5 to -6

A similar experiment was undertaken on 22 January 1982 with leaf tissue collected from the same provenances at the same location. Results were highly variable because of lack of hardiness and marked differences in stage of leaf development at this time of year, even though care was taken to select fully expanded mature leaves and randomly allocate them among temperature treatments. The frost tolerance tests at this time of year resulted in a different rating of the provenances as listed below in increasing order of damage.

	Provenance	Altitude
1.	No. 17 N.S.W.	1210-1300
2.	No. 38 Ben Nevis, Tas.	820
3.	No. 35 Hartz Mt., Tas.	560
4.	No. 44 Luina, Tas.	450
5.	No. 55 Bicheno, Tas.	460

It is interesting to note that this ranking also lists the provenances in decreasing order of altitude of their seed source. Whether this relationship holds for other provenances of varying altitude during the unhardened actively growing state requires further testing. If the relationship holds, selection of seed for resistance to unseasonable frosts as occurs on regenerated land in northeastern Tasmania during the growing season would be possible.

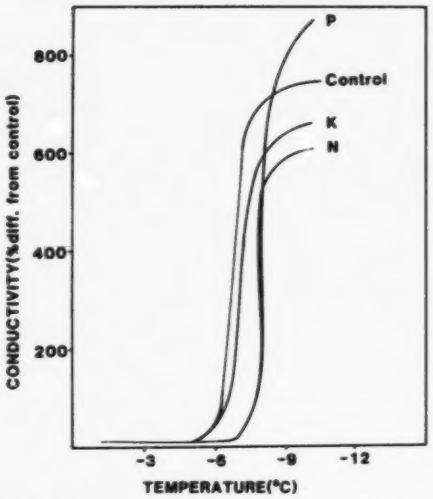
c) Effects of nutrient addition on frost tolerance of E. delegatensis

The effects of fertilization on frost tolerance of woody plants is not well documented. Increased addition of nitrogen during the latter part of the growing season can increase susceptibility to frost of northern temperate species that show a strong negative correlation of shoot growth to frost tolerance (Colombo et al. 1982). However, in the case of eucalypts, it would appear that the effects of nutrient addition on frost tolerance have not been examined. In association with a trial examining the effects of nitrogen, phosphorus and potassium additions to Camden granitic soil on **E. delegatensis** seedling growth, frost tolerance was also examined in leaf tissue from the various NPK treatments at various levels of $CaCO_3$ addition (0, 0.3, 1.0 and 2.5 g) per pot. Addition of N and P (over all levels of $CaCO_3$) increased frost tolerance (Fig. E5); frost damage began at -7°C in comparison with -6°C with control and potassium-treated seedlings.

The addition of $CaCO_3$ to Camden soil to increase soil pH resulted in increased frost tolerance as assessed by the conductivity method (Fig. E6). It would appear that calcium addition possibly reduces electrolyte leakage by stabilizing cell membranes.

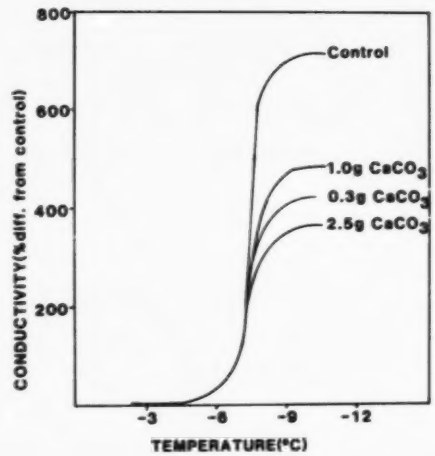
Frost tolerance did not appear to be related to seedling growth rates as was generally observed in northern temperate species (Colombo et al. 1982). Similar results were found by Paton (1972) in **E. viminalis.** In this experiment the highest growth rates were observed in the nitrogen and potassium addition treatments.

These data indicate that the nutritional status of the plant can markedly affect its frost tolerance; however, further investigation is necessary to verify and explain the mechanisms involved.



FROST HARDINESS OF E.delegatensis SEEDLINGS GROWN IN CAMDEN SOIL WITH ADDED N, P OR K

Figure E5. Effect of temperature on electrolyte leakage of **E. delegatensis** leaf tissue from seedlings grown in Camden soil with added nitrogen (N), phosphorus (P) or potassium (K).



FROST HARDINESS OF E.delegatensis SEEDLINGS GROWN IN CAMDEN SOIL WITH ADDED $CaCO_3$

Figure E6. Effects of temperature on electrolyte leakage from **E. delegatensis** leaf tissue fom seedlings grown in Camden soil with added calcium carbonate.

SECTION F: CONCLUDING DISCUSSION

In field experiments conducted in northeastern Tasmania, Camden Plateau, Compartment 49, during 1981-1982, significant increases in shoot growth of **E. delegatensis** were obtained in both single-tree plots of existing 6-year-old regeneration and planted, nursery-grown seedlings in response to weed control treatments (manual weeding and herbicide application) that removed competing vegetation. Site preparation treatments caused measurable changes in soil temperature, soil moisture, and available nitrogen. The marked growth response of the shallow-rooted transplanted seedlings could be accounted for by a combination of these factors, of which increased soil moisture following the removal of competition (mainly **Poa** grass) was probably the most important. In contrast, site preparation treatments in the single-tree plots generally gave a much smaller growth response. The observed changes in soil temperature, moisture and available nitrogen had no significant effects on water relations of the more deeply rooted, established 6-year-old trees. Obviously moisture was not limiting even during the drier summer months. In this case it would appear that the observed temperature increase of between 5 and 6°C in the rooting zone, increases in available nitrogen, and possibly other undetermined factors could account for the significant increases in shoot growth observed between weed control treatments.

It would appear that, if grass competition develops before the seedlings have formed a deep root system, the seedlings can be expected to suffer summer drought even in areas of high rainfall. Part of the beneficial effect of a hot regeneration burn or of mechanical cultivation, therefore, is to delay development of a grass sward until the seedlings have established a deep root system. Nevertheless, subsequent development of the sward would then result in lower temperatures in the root zone and a reduction in the availability to the trees of N and other nutrients. Both these factors will reduce growth.

The two very damaging frosts that occurred during the growing season make it clear that frost is almost certainly a major factor causing growth check. During the following winter (1982) screen minima of -5°C or lower occurred on nine nights between 20 May and 23 September. On 19 July a screen temperature of -8°C was recorded. Corresponding ground temperatures were probably as low as -10°C. Extensive kill of the fern **Polystichum proliferum** was noted and several of the manually weeded established trees died, most probably from frost heave and freezing damage in the partly exposed root system. Temperatures were low enough to kill even hardened eucalypt buds at screen height, and leaves were killed within 30 cm of ground level.

The overall influence of frost, for which no control treatment could be provided in the field, made it difficult to assess the relative importance of the other factors examined.

The marked response of seedlings to N supply under conditions of abundant moisture and uniform temperature was demonstrated in the pot experiments. That a decrease in temperature of the root zone would be detrimental to growth is a reasonable assumption, although it has not been demonstrated specifically for E. **delegatensis.**

Any factor that reduces the rate of height growth of the seedlings prolongs the length of time for which they remain in the cold inversion layer that develops at ground level during nocturnal radiation. Such factors, therefore, increase the likelihood of frost damage.

There is no evidence to indicate that allelopathic interaction occurs between grass and tree seedlings. On the other hand, the presence of tree seedlings appears to stimulate the development of grass roots quite markedly, and thus probably to enhance the ability of the grass to compete for water, nutrients, and growing space.

Current information suggests that growth check is induced in part by physical competition for soil water and nutrients and in part by the altered microclimate in deforested areas on the Camden Plateau, which contributes markedly to the occurrence of unseasonable frosts to which E. **delegatensis** seedlings are particularly susceptible. The additional overriding stress imposed by frequent frosts appears to reduce vigor and increase susceptibility to insect and fungal attack.

From the foregoing discussion it is clear that an ideal method of regeneration would provide protection from frost, discourage the proliferation of grass, and improve the supply of moisture and nutrients to seedlings during initial establishment. Many of these desirable effects could probably be achieved in part with a uniform shelterwood system, in which burning of an understorey (of shrubs that had already suppressed grass) or mechanical cultivation is carried out either before or after the initial felling. Variants of the uniform system in which groups of trees are felled or retained might also be successful. With a group system, though, there is the possibility that the dimensions of a gap left in the canopy will be such as to form an effective frost hollow and accentuate the problem.

For the regeneration of clear-felled areas the options are rather unsatisfactory. Many checked seedlings have been observed eventually to achieve normal growth. However, the rotation will be increased by as much as 20 years, stocking will be irregular, and the form of many trees is likely to be poor. Planting, accompanied by intense initial site preparation, is another option. The variation in frost tolerance among provenances offers some scope for improving the frost resistance of planting stock. Frost resistance may also be improved by the nutritional regime used in raising the seedlings. In view of the importance of radiation frost damage in the air layer 0-30 cm above ground, consideration should also be given to planting large potted seedlings, especially where the objective is to begin afforestation of extensive treeless grass plains. Another possible option is to establish a "nurse" crop beneath which the eucalypts could grow protected from frost. Silver wattle is a likely nurse species, although this, too, may be difficult to establish on treeless areas.

Suggested topics for future work

1) The effect of root zone temperature on seedling growth of both E. **delegatensis** and **Poa** grass.

2) The nature of the relationships between frost tolerance, nutrition, and drought tolerance in eucalypt seedlings.

3) The phenology of root growth of eucalypt seedlings and grass **in situ** .

4) Comparison of the nutritional and water relations of eucalypt seedlings and grass.

5) Establishment of semi-operational experiments involving removal of grass (by herbicide or cultivation), fertilizer treatment, and stand manipulation in order to develop silvicultural techniques to alleviate growth check.

Conclusions

1) Growth check is common on areas above 800 m, especially where **Poa** grass is common.

2) Recurring frost, if it does not kill, curtails growth and keeps seedlings within a layer prone to radiation frost for a long period. This effect is enhanced by the presence of **Poa** grass.

3) **Poa** grass proliferation after clear-cutting, and by virtue of root growth that is superior to that of E. **delegatensis,** competes successfully with seedlings for soil moisture, mineral nitrogen, possibly other nutrients, and growing space.

4) **Poa** grass does not appear to interact allelochemically with tree seedlings nor does it inhibit nitrogen mineralization processes by such means.

5) A silvicultural system that (a) reduces the effect of frost, (b) reduces grass competition, (c) enhances nitrogen status of the soil should improve growth and survival of E. **delegatensis** seedlings. Therefore, uneven-aged stand management (shelterwood, group selection), together with encouragement of a cover crop or understorey of legumes, in particular **Acacia** and **Pultenaea,** could prove to be effective.

LITERATURE CITED

ANSTETT, A. 1956. Sur une méthode rapide de détermination du rapport C/N dans les sols, les amendments organiques et les végétaux. Proc. VI Congr. Sci. Sol. (Paris), p 693-699.

ARONSSON, A. and ELIASSON, L. 1970. Frost hardiness in Scots pine (**Pinus silvestris** L.). 1. Conditions for test on hardy plant tissues and for evaluation of injuries by conductivity measurements. Stud. For. Suec. 77. 30 p.

ATKINSON, D. 1977. Some observations on the root growth of young apple trees and their uptake of nutrients when grown in herbicided strips in grassed orchards. Plant and Soil 49:459-471.

AUDUS, L.J. 1946. A new soil perfusion apparatus. Nature 148:419.

BARRITT, N.W. 1933. The nitrification process in soils and biological filters. Ann. Appl. Biol. 20:165-184.

BREMNER, J.M. 1965. Inorganic forms of nitrogen. p. 1179-1237 in C.A. Black et al., **Ed.** Methods of soil analysis. Amer. Soc. Agron., Madison, Wis.

BROADBENT, F.E. 1965. Effect of fertilizer nitrogen on the release of soil nitrogen. Soil Sci. Soc. Am. Proc. 29:692-696.

BROWN, R.W. 1971. Determination of leaf osmotic potential using thermocouple psychrometers. p. 198-209 in Psychrometry in Water Relations Research. R.W. Brown and B.P. Van Haveren, **Ed.** Proc. Symp. on Thermocouple Psychrometers, Utah State Univ. 17-19 March 1971.

BURKE, M.J., GUSTA, L.V., QUAMME, H.A., WEISER, C.J. and LI, P.H. 1976. Freezing injury in plants. Ann. Rev. Plant Physiol. 27:507-28.

CHAPMAN, H.D. 1965. Cation-exchange capacity. p. 891-901 in C.A. Black et al., **Ed.** Methods of soil analysis. Amer. Soc. Agron., Madison, Wis.

CHASE, F.E., CORKE, C.T., and ROBINSON, J.B. 1968. Nitrifying bacteria in soil. p. 573-611 in T.R.G. Gray and D. Parkinson, **Ed.**, The ecology of soil bacteria. Univ. Toronto Press.

COLOMBO, S.J., WEBB, D.P. and GLERUM, C. 1982. Cold hardiness and bud development under short days in black and white spruce seedlings. p. 171-176 in J.B. Scarratt, C. Glerum and C.A. Plexman, **Ed.** Proceedings of the Canadian Containerized Tree Seedling Symposium. Dep. Environ., Can. For. Serv., Sault Ste. Marie, Ont. COJFRC Symp. Proc. O-P-10.

CONWAY, E.J. 1947. Microdiffusion analysis and volumetric error. Crosby Lockwood, London.

DEXTER, S.J. 1932. Studies of the hardiness of plants: a modification of the Newton pressure method for small samples. Plant Physiol. 7:721-726.

ELLIS, R.C. 1964. Dieback of alpine ash in north-eastern Tasmania. Austr. For. 28:75-90.

ELLIS, R.C. 1971. Dieback of alpine ash as related to changes in soil temperature. Austr. For. 35:152-163.

ELLIS, R.C. 1974. The seasonal pattern of nitrogen and carbon mineralization in forest and pasture soils in southern Ontario. Can. J. Soil Sci. 54:15-28.

ELLIS, R.C., MOUNT, A.B., and MATTAY, J.P. 1980. Recovery of **Eucalyptus delegatensis** from high altitude dieback after felling and burning the understory. Austr. For. 43: 29.

FLINT, H.L., BOYCE, B.R. and BEATTIE, D.J. 1967. Index of injury - A useful expression of freezing injury to plant tissues as determined by the electrolytic method. Can. J. Plant Sci. 47:229-230.

GEE, G.W. and FEDERER, C.A. 1972. Stomatal resistance during senescence of hardwood leaves. Water Resour. Res. 8:1456-1460.

GREEN, L.M. and WARRINGTON, I.J. 1978. Assessment of frost damage in radiata pine seedlings using the diffusate electroconductivity technique. N.Z. J. For. Sci. 8:344-350.

GROVES, R.H., KERAITIS, K. and MOORE, C.W.E. 1973. Relative growth of **Themeda australis** and **Poa labillardieri** in pots in response to phosphorus and nitrogen. Austr. J. Bot. 21:1-11.

HEILMAN, P. 1975. Effect of added salts on nitrogen release and nitrate levels in forest soils of the Washington coastal area. Soil Sci. Soc. Amer. Proc. 39:778-782.

JONES, J.M. and RICHARDS, B.N. 1978. Fungal development and the transformation of ^{15}N-labelled amino-acid and ammonium-nitrogen in forest soils under several management regimes. Soil Biol. Biochem. 10:161-168.

JOHNSON, D.W., EDWARDS, N.T., and TODD, D.E. 1980. Nitrogen mineralization following urea fertilization of a forest soil under field and laboratory conditions. Soil Sci. Soc. Am. J. 44:610-616.

KANEMASU, E.T., THURTELL, G.W. and TANNER, C.B. 1969. Design, calibration and field use of a stomatal diffusion porometer. Plant Physiol. 44:881-885.

LEES, H. and QUASTEL, J.H. 1946. Biochemistry of nitrification in soil. Biochemistry. 40:803-828.

LETESTU, S., Ed. 1966. International Meteorological Tables. World Meteorol. Org., Geneva. No. 188. TP 94.

LEVITT, J. 1972. Responses of plants to environmental stresses. Academic Press, New York, 697 p.

MOORE, C.W.E., and KERAITIS, K. 1971. Effect of nitrogen source on growth of eucalypts in sand culture. Austr. J. Bot. 19:125-141.

MURPHY, J., and RILEY, J.P. 1962. A modified single solution method for the determination of phosphate in natural waters. Anal. Chim. Acta. 27:31-36.

NAMBIAR, E.K.S. and ZED, P.G. 1980. Influence of weeds on the water potential, nutrient content and growth of young radiata pine (**Pinus radiata**). Austr. For. Res. 10:279-288.

O'CONNOR, K.F., ROBINSON, J.B. and JACKMAN, R.H. 1962 Bacterial conditions and nutrient availability in a tussock grassland soil under different cultural treatments. p. 177-182 in International Society of Soil Science, Transactions Joint Meeting of Commissions IV & V. Palmerston North, New Zealand.

O'CONNOR, K.F., ROBINSON, J.B., and CORKE, C.T. 1966. Nitrification in soils of Magallanes Province, Chile, in relation to vegetation conditions and land development practices. Monograph 1. Progressos en biologia del suelo. Bahia Blanca Argentina, Montevideo.

OLSEN, S.R. and DEAN, L.A. 1965. Phosphorus. p. 1035-1049 in C.A. Black et al., Ed. Methods of soil analysis. Amer. Soc. Agron., Madison, Wis.

ORME, R.K. 1971. Regeneration of eucalypt forests, Surrey Hills, Tasmania. Univ. Tasmania, Masters Thesis.

OVERREIN, L.N. 1970. Tracer studies of nitrogen immobilization-mineralization relationships in forest raw humus. Plant and Soil 32:478-500.

PATON, D.M. 1972. Frost resistance in Eucalyptus: a new method for assessment of frost injury in altitudinal provenances of E. **viminalis**. Austr. J. Bot. 20:127-39.

PATON, D.M. 1981. Eucalyptus physiology. III. Frost resistance. Austr. J. Bot. 29:675-88.

PEREIRA, J.S. and KOZLOWSKI, T.T. 1976. Leaf anatomy and water relations of **Eucalyptus camaldulensis** and E. **globulus** seedlings. Can. J. Bot. 54:2868-2880.

PURCHASE, B.S. 1974. The influence of phosphate deficiency on nitrification. Plant and Soil 41:541-547.

QUAMME, H., WEISER, C.J. and STUSHNOFF, C. 1973. The mechanisms of freezing injury in xylem of winter apple twigs. Plant Physiol. 51:273-77.

RICHARDS, L.A. 1965. Physical condition of water in soil. p. 128-152 in C.A. Black et al., Ed. Methods of Soil Analysis. Amer. Soc. Agron., Madison, Wis.

RICHARDSON, H.L. 1938. The nitrogen cycle in grassland soils: with special reference to the Rothamsted Park Grass experiment. J. Agric. Sci. 28:73-121.

RICHARDSON, S.D. 1953. Root growth of **Acer pseudoplatanus** L. in relation to grass cover and nitrogen deficiency. Mededelingen van de Landbouwhogeschool te Wageningen, Nederland. 53:75-97.

RITCHIE, G.A. and HINKLEY, T.M. 1975. The pressure chamber as an instrument for ecological research. Adv. Ecol. Res. 9:165-254.

ROBINSON, J.B. 1963. Nitrification in a New Zealand grassland soil. Plant and Soil 19:173-183.

SABEY, B.R., FREDERICK, L.R. and BARTHOLOMEW, W.V. 1959. The formation of nitrate from ammonium nitrogen in soils. Soil Sci. Soc. Amer. Proc. 23:462-465.

SUTTON, R.F. 1968. Ecology of young white spruce (**Picea glauca** [Moench] Voss). Can. Dep. For. Rur. Devel., For. Res. Lab., Ont. Reg. Intern. Rep. 0-10.

THERON, J.J. 1963. The mineralization of nitrogen in soils under grass. S. Afr. J. Agric. Sci. 6:155-164.

THOMAS, D.H. ad BARBER, H.N. 1974. Studies of leaf characteristics of a cline of **Eucalyptus urnigera** from Mount Wellington, Tasmania. I. Water repellency and the freezing of leaves. Austr. J. Bot. 22:501-12.

TIMMIS, R. 1976. Methods of screening tree seedlings for frost hardiness. p. 421-35 in M.G.R. Cannell and F.T. Last, Ed. Tree Physiology and Yield Improvement. Academic Press, London.

VAN DEN DRIESSCHE, R. 1969. Measurement of frost hardiness in 2-year-old Douglas-fir seedlings. Can. J. Plant Sci. 49:159-172.

WARRINGTON, I.J. and ROOK, D.A. 1980. Evaluation of techniques used in determining frost tolerance of forest planting stock: A review. N.Z. J. For. Sci. 10:116-32.

WEBB, D.P. and VON ALTHEN, F.W. 1979. Establishment of high quality hardwoods in open field sites in southern Ontario. p. 177-189 in H. Oswald Ed. Symposium on establishment and treatment of high quality hardwood forests in the temperate climatic region. Proc. IUFRO, Nancy-Champenoux, France, 11-15 Sept. 1978.

WEBB, D.P. and VON ALTHEN, F.W. 1979. No allelopathic interference found between living quackgrass and 2-year-old sugar maple seedlings. Dep. Environ., Can. For. Serv., Ottawa, Ont. Bi-mon. Res. Notes. 35(1):1-2.

WILLIAMS, B.L. 1972. Nitrogen mineralization and organic matter decomposition in Scots pine humus. Forestry 45:177-188.

WILNER, J. 1960. Relative and absolute electrolytic conductance tests for frost hardiness of apple varieties. Can. J. Plant Sci. 40:630-37.